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What future for postdoctoral fellows?

Are postdoctoral fellows the lowest form of academic life? This belief, common enough, is well supported by the evidence now gathered by the Grodzins committee (see page 443). Postdoctoral fellows are badly paid by comparison both with PhD graduates who have chosen other careers and, more galling, with those already lucky enough to have found permanent academic posts. The conditions of employment — sometimes the lack of medical insurance or of retirement benefits — are often demeaning, yet postdoctoral fellows shoulder a disproportionate share of responsibility for academic research programmes which permit the building of an independent reputation only when circumstances are right. So why do 12,500 scientists in the United States put up with these indignities? Classically, the prize has been a tenured position at a good university. The chance of that is no longer high, at least in the more crowded fields (biology in particular). So postdoctoral fellows have a growing sense, often just, of being exploited.

So is this scandal? The Grodzins committee rightly shrinks from giving the simple answer yes to this oversimple question. To be sure it would be outrageous, if true, that self-governing bodies of academics — American universities — should deprive their temporary colleagues in research of medical insurance and the like. But the American system of postdoctoral fellowships is different both in conception and in its present function from some of the systems in place elsewhere. The intention, adapted from the old German system of the *Privatdozent*, is that postdoctoral fellows should be apprentice academics — people of promise who are blessed with a few years in which to establish independent reputations as scholars. At the turn of the century in Germany, such people were paid less well than established academics, chiefly because the universities were chronically short of funds. In the United States, the system has never been strictly comparable with the German, if only because American universities have mostly relied on outside sources for funds with which to support the bulk of postdoctoral fellows. In the past few decades, the system has been further unsettled by rapid expansion, especially in biology, and by the relative decline of academic posts. The avoidance of all disappointment is impractical. What else should be done?

The most urgent need is that universities should take more direct responsibility for their postdoctoral fellows. If the implicit promise of such appointments — a tenured teaching post — has to be broken, the university cannot decently shrug its shoulders and let the disappointed fellow walk away. Although people now embark on fellowships like these with their eyes wide open to the risks, after three years or so and the accretion of personal as well as professional responsibilities, a disappointed fellow needs more than an expression of sympathy. (Pay during the tenure of a fellowship matters less.) The most constructive if the most nebulous of the recommendations of the Grodzins committee is that universities should establish continuing machinery within which recurring problems can be discussed. The underlying need is that universities should acknowledge both the dependence of their reputations in research on the floating population of postdoctoral people and the need that, if a stint should end in disappointment, there should be some tangible means by which a postdoctoral fellow can thereafter make his way in the world.

The problem is not merely a problem for universities in the United States. Over the past several years, in most of the university systems in which research is (in practice, as well as theory) an important part of everybody's work, it has turned out to be expedient that PhD graduates should be the backbone of the

research effort in many academic departments. Broadly speaking, they are able (intelligent and skilled) and willing. Not all people with these qualifications are formally included in the category of postdoctoral fellow used by the Grodzins committee, which technically excludes those whose salaries are provided by short-term research contracts but also, for example, people elected with considerable solemnity to junior fellowships at Harvard University and their equivalents elsewhere.

In countries such as France, the universities in partnership with organizations such as the Centre National de la Recherche Scientifique have sought to solve the difficulties which arise by means of essentially permanent appointments to research posts in parallel with regular academic posts. The snag is that too few young people enter research, and that dead wood accumulates. Elsewhere, in Britain for example, the embarrassment caused by the frequent need to tell people living off soft money that there will not be an extension has led to the convention among grant-making agencies that after one or at the most two stints, postdoctoral workers should be told that, however talented, they are ineligible for further support; this is a device for making the problem go away, not for solving it. The American practice, more tolerant of repeated extensions of postdoctoral fellowships, has the effect of postponing the same problem. The risk is that the brightest people will be among the first to leave.

The Grodzins committee can in the circumstances be forgiven for having very little to offer as a remedy for the circumstances that have arisen in the United States. Nowhere have the problems of postdoctoral employment been dealt with easily. The committee's proposal that there should be a system of federally-financed postdoctoral fellowships in the original mould of academics-in-waiting would help, but not much. So long as the universities need postdoctoral fellows to assist with their research programmes, and for as long as grant-making agencies consider that posts of this kind provide valuable post-postdoctoral training — and can afford to pay the stipends — the creation of new fellowships will continue. The dramatic expansion in the biological sciences in the past few years will and should continue, for there is plainly no end in sight to the biological projects that can profitably be undertaken. Almost by accident, however, the new Administration's budget may change the shape of the expansion. By outlawing the contributions the National Institutes of Health have been making to the universities receiving postdoctoral fellows, the Administration has probably ensured that more postdoctoral fellowships will be paid out of project grants, not from a dedicated budget. That will at least help to reduce unreasonable expectations of continued academic work.

The other steps that need to be taken are more practical, and humdrum. Even when postdoctoral fellows have embarked on their careers knowing what the risks may be, their eventual departure from the university may be a hardship, even an injustice. The job market in the field concerned may have changed, or the point at which a contract ends may be one at which it is exceedingly improbable that a suitable job elsewhere might be found. So is there not a case for asking that universities (rather than the sponsoring agencies) should be able to soften the problems of this inevitably painful transition period? Several stratagems are possible. The ideal would be that postdoctoral fellows at the end of their contracts and unable then to find a suitable job should be paid a bounty, a kind of retrospective recompense for the deprivations of earlier years. At the Massachusetts Institute of Technology, a scheme for providing



short but limited spells of continued employment (at the expense of the institution) is being considered. Neither of these schemes is ideal. All schemes for easing the transition from academic life to the outside world will leave much to be desired (for not all disappointment is avoidable) — and will cost money. But the Grodzins committee shows clearly enough that something needs to be done. It is unseemly that the academic community, while rightly insisting on the highest standards of scholarship and achievement from candidates for permanent faculty positions, can as casually as at present shrug off its responsibilities for those who, for one reason or another, fail to be chosen.

Change wanted

The University of London, in the throes of reorganization or at least of the contemplation thereof for the past year, will not be much helped by the second interim report from the Swinnerton-Dyer Committee, which has been charged with suggesting how student numbers might be reduced and the cost of operations still more rapidly reduced. For what the committee says (see page 447), while sensible and moderate enough, provides neither a prescription with which the several components of the university will be unable to argue nor a means by which they will sense an incentive to reorganize themselves. The university has a long, disputatious summer ahead of it.

What is happening in London is nevertheless a foretaste of what is likely to be happening in other British universities a few weeks from now, when the University Grants Committee translates into putative grants for individual universities the dire threats it has been making of hard times ahead. The university differs from the other universities in Britain, except the University of Wales, in being a federal institution. For much of the past fifteen years, the various London colleges have become more and not less autonomous as the federal centre has shed responsibility for extraneous tasks such as the supervision of academic standards in associated institutions (many of which have become universities in their own right) and the provision of public examinations in much of the British Commonwealth. The separate colleges of the university with responsibility for teaching undergraduates are now almost autonomous in awarding degrees. All of the colleges and most of the specialized institutes of the university employ their own members of staff. With the exception of Imperial College, which has a direct relationship with the University Grants Committee, the several components of the university are financed by means of grants from what is called the "court" of the university, which divides whatever it can spare according to rules and formulae which are not made known, even to the recipients of its cheques.

The prospect, as described by the committee, is daunting. In the course of the three years ahead, income will be reduced by 15 per cent and the committee has taken the sensible view that economizing on equipment and other materials has already gone far enough. So the threatened cut implies smaller payrolls, reducing more quickly than natural wastage will permit. With that starting point and the implicit assumption that academic staffs and their ancillaries will be reduced in equal proportions, the committee arrives naturally at its conclusion that at least one college must be disbanded, and that there should be a system of peer review for deciding which academic departments elsewhere deserve the university's support. This is what people within the university will be arguing about. Why Chelsea College for oblivion? Why not somewhere else? And so one.

The university will be a less wretched place in the months ahead if it can concentrate instead on the more constructive parts of the committee's report. On the proper grounds that it would seem desperately unfair that all teachers of say, psychoastrometry in one of the London colleges should lose their jobs because the process of peer review had decided that the teaching of this subject should be concentrated elsewhere within the university, the committee argues that there should be an attempt to make the university rather than its separate parts the ultimate employer of

all the university's academics. Then, the argument goes, it would be possible to ensure that even if it seems sensible to concentrate the teaching of psychoastrometry in some larger department of astrometry on some other site, psychoastrometry will still be taught by the best psychoastrometrists. The argument is unshakable while, in their present panic, the university's academics would probably agree that their contracts of employment should be transferred to an employer of the last resort, the university.

The university must also grasp the more painful nettle of deciding which kinds of employees it hopes to keep on its books. The assumption that academic and non-academic staff must be reduced in step is genteel but not essential. The peer review of academic performance that the Swinnerton-Dyer Committee asks for will take time, and the outcome cannot be conclusive. A managerial review of the necessity of the armies of ancillary staffs employed by the university could be quicker and might prove a more effective economy. It would naturally be wrong to think that an academic institution such as the largest university in the United Kingdom could be run entirely by its academics, but is there not a case for thinking that fewer cleaners, porters and even technicians might be prudent in parlous times? The academics in the University of London, as elsewhere, will shrink from this question, some of them because they cannot imagine how their own energies might be bent to deciding what denomination of stamp should be stuck to what kind of letter, most of them because they would consider the enquiry inequitable as between academics and others. But is not the university an institution whose chief purpose is academic?

Some silver lining

In the United States as elsewhere except France — for the time being — professional people are adjusting to smaller public subventions for research. The cause is everywhere the same: inflation. By now, it is well-established that the threat posed by inflation to civilizing institutions (universities, research laboratories, even entrepreneurial businesses) is unavoidable even if, for a time, insidiously unrecognized. Occasionally, however, some casualties of the attempts by governments to offset inflation by reducing spending are not losses at all. So much is illustrated by the new Reagan budget, widely and justly bemoaned in the United States. Although the new Administration appears to have acted over the whole range of public policy without stopping to calculate the consequences (in which it resembles the Thatcher government's policy towards British universities) some of the uncalculated consequences will be welcomed. The following are some examples.

President Carter's budgets for the National Science Foundation has been left unchanged by President Reagan except in one respect — the foundation's educational budget was cut by a half, that for the support of the social sciences virtually eliminated and that for international activities reduced. Tactically, the Administration has been clever. Only a small part of the foundation's constituency will have been hurt so badly that it squeals aloud. The threatened areas are also those in which the National Science Foundation has been living dangerously. Nobody can now claim that the curriculum development the foundation has been sponsoring is pointed and effective; rather, too little resources are being spread too thinly. And although it is unforgivable (and will not be forgiven in Europe) that the Administration should have cut the foundation's international budget without prior consultation, there is at least some merit in asking (as many in Washington are doing) whether continued support for the International Institute for Applied Systems Analysis in Vienna, to the tune of nearly \$3 million a year, is the best use for such a sum of money. None of this implies that the Reagan cuts are to be welcomed for the hardship they will bring and the damage they will do. The moral, if there is one, is that if times ever improve, people should discriminate more carefully between good projects and the not so good.

Anxiety about postdoctoral prospects

Academy report sees trouble in store

Washington

A postdoctoral position may be a necessary but is no longer a sufficient stepping stone to a tenured academic position in the United States, according to a report now published by the National Research Council, the research arm of the National Academy of Sciences. Ironically, the council was one of the first agencies to operate a system of postdoctoral fellowships soon after its own creation in 1916.

The report, commissioned by the National Science Foundation and prepared by a committee under Dr Lee Grodzins of the Massachusetts Institute of Technology, starts from the view that postdoctoral fellowships make an invaluable contribution to research, and remain a valuable preparation for academic life. It is dismayed by the mismatch between the growth in the 1970s of the numbers of PhD graduates employed on postdoctoral grants and the decline of openings in tenured faculty positions.

In 1979, there were an estimated 12,500 postdoctoral fellows working in scientific fields, some 5 per cent of the total doctoral labour force in United States universities and an increase of 50 per cent compared with 1972. Roughly a fifth of postdoctoral positions in 1979 were in government laboratories and other such institutions.

The biosciences forced the pace of expansion in the 1970s, accounting for 7,300 postdoctoral fellows in 1979. The committee cites federal policies as the driving force and the increasing proportions of new PhD graduates seeking postdoctoral positions (62.7 per cent in 1979) and the increased duration of postdoctoral stints as ancillary causes. By contrast, the numbers of postdoctoral fellows in physics and chemistry (1,100 and 1,700 respectively in 1979) are declining both in absolute numbers and as proportions of all postdoctoral fellows or of those obtaining PhDs in physics and chemistry. In engineering and mathematics, postdoctoral fellows in 1979 numbered only 400 and 200 respectively, a mere 1 per cent of the total numbers in academic posts (not all tenured).

The career benefits of a spell as a postdoctoral fellow are mixed. Data for the employment of the 1972 cohort of PhDs suggest that graduates make an early choice between a postdoctoral stint at one of the 59 "major research universities" (defined as the institutions with two-thirds of all research budgets in higher education) and a

tenured post elsewhere. Thus only 5 per cent of those graduating in 1972 in the biological sciences and electing for a postdoctoral stint had found tenured positions at research universities, but 28 per cent of those deciding otherwise had by then found tenured positions elsewhere (while 9 per cent had found posts at the major universities).

Hiring practices vary considerably from one field to another. In the major research universities, 88 per cent of the most recently appointed assistant professors in chemistry had done a postdoctoral stint, compared with 82 per cent in physics, 73 per cent in the biological sciences and only 29 per cent in engineering.

There is some evidence that a postdoctoral stint can mean a lasting loss of salary. Even in tenured faculty posts, the median salaries of those with postdoctoral experience (from the 1972 cohort) are between \$1,000 and \$2,000 less than those of direct entrants. The differences are even

larger for those who have found jobs in government service and industry. Most people seem to have had to extend their postdoctoral stints by at least a year while seeking suitable jobs, and there is now some evidence that new PhDs are less inclined to look for postdoctoral appointments — the percentages of PhD graduates seeking postdoctoral appointments have been declining since the early 1970s in chemistry and physics, and appear to have reached a plateau (above 60 per cent) in the biological sciences.

The report resembles others in its evidence that women and members of minority groups are at a disadvantage both in finding postdoctoral appointments and in profiting from them. In 1979, overseas scientists accounted for 38 per cent of all postdoctoral appointments and for 70 per cent of postdoctoral appointments in engineering. The report says nothing about the numbers of US citizens holding postdoctoral positions overseas.

Low pay, indignities and uncertainty

Washington

Among postdoctoral fellows in the United States, those working in the biological sciences are the most numerous, the most eager and the most at the mercy of the job market. Of PhDs in the biological sciences, 58 per cent take on a postdoctoral stint (compared with 50 per cent in physics). Half of biology postdoctoral fellows hold their appointments for two years or more and 43 per cent of them have to prolong their stints while looking for jobs. (Both chemistry and physics are under more pressure, with 38 per cent and 36 per cent respectively.)

Postdoctoral stipends are lower in biology than in other fields except psychology and the social sciences, at an average of a mere 57 per cent of tenured academics of the same seniority. (Mathematician postdocs can expect to earn 96 per cent of an academic salary.) Biologists on fellowships also make up the largest proportion in their field of all research personnel (16 per cent) and of equivalent full-time research staff (27 per cent) in major research universities.

The Grodzins report, *Postdoctoral Appointments and Disappointments* (National Academy of Sciences, \$17.75), justifies the second part of its title with anecdotal as well as statistical information gathered by a questionnaire to graduating PhDs. Postdoctoral stipends seem to have been a constant source of complaint. One said that the postdoctoral position he turned down seemed to be "little more than a glorified graduate student", another that postdoctoral fellows are not "treated as professionals", earning as they do less than "laboratory technicians, some

secretaries, janitors, etc".

There appears to be a strong feeling among biology postdoctoral fellows that the "pay-back" provisions of fellowships awarded by the National Institutes of Health are iniquitous. NIH fellows are required to refund part of their stipends if they do not match the duration of their fellowships with an equal time in some other health service field. One fellow compared this condition with "indentured service", another (a woman) pointed out that the requirement would conflict with her family life.

Many postdoctoral fellows acknowledged the opportunities provided for getting on with personal research, sometimes as a prelude to a complaint of having been "exploited" as "second class citizens". The lack of medical insurance and retirement provision appears to be resented as demeaning. The report quotes an eloquent appeal from a mathematician for more but more rewarding postdoctoral fellowships in his field, and an engineering professor notes that it is now almost impossible to persuade American citizens to take fellowships because of the high salaries in private industry.

Many respondent PhDs told the committee that they regarded postdoctoral fellowships as a "holding pattern for the academic life" — but the committee says there is no evidence that universities are losing the brightest people because of the uncertainties and indignities involved. Employers outside the university sector differ in their estimations of the value of postdoctoral experience — government laboratories welcome postdocs, private industry sometimes finds them inflexible.

On the grounds that postdoctoral positions are increasingly being used to support academics in waiting, the Grodzins committee urges that the federal government should establish a system of postdoctoral awards (perhaps 250 new awards a year) at stipends half as much again as awards now available, that there should be roughly 50 similar awards for overseas scientists and that universities should give more thought to the careers of their postdoctoral fellows, setting up oversight committees for the purpose. Fearing employment difficulties for postdoctoral people, the committee also asks that the National Science Foundation extend the scope of its present demographic work to cover postdoctoral fellows.

US minerals

Resource warcries

Washington

Is the Soviet Union conducting an undeclared "resource war" against the West in an effort to jeopardize supplies of minerals needed for high technology products such as computers and military aircraft? Yes, according to conservative foreign policy groups such as the Washington-based Center on Economics and National Security, claiming that recent Soviet moves to increase purchases of so-called critical minerals on the international market is part of a broader strategy to weaken the West's military defences.

Not so, according to a group of geologists, mining engineers and assorted Soviet watchers who met last month at the Johns Hopkins University's School of Advanced International Studies. Although agreeing that the Soviet Union had, indeed, stepped up its purchases of critical minerals and reduced its exports, the meeting found little evidence that this was part of a deliberate military strategy, but rather reflected the Soviet Union's difficulties in meeting its own mineral needs.

The debate is not merely academic — for example the concept of a "resource war" is already being used by legislators such as Representative Jim Santini of Nevada to justify attempts to open up federal lands in the United States for exploration by mining companies, a move firmly opposed by environmentalists. Similar arguments are being used to the same ends by the new Interior Secretary, Mr James Watt.

There is little debate on the principal facts of mineral supply. The United States is almost totally dependent on foreign countries for many of the minerals used in high technology products, such as the manganese necessary for specialty steels (of which South Africa is the main supplier), and the cobalt needed in jet engine alloys (93 per cent of which is imported from Zambia and Zaire).

In theory, this makes the United States highly vulnerable to cartel-type actions such as those imposed by the oil-producing

countries in the early 1970s. In practice, policy-makers are having to decide how likely such actions might be, and what could be done to minimize the chance of dislocation, for example by developing substitute materials.

The new Administration has started on a much more hawkish note than its predecessor. Secretary of State Alexander Haig, as president of United Technologies, had previously talked of "resource battlegrounds" in the Third World. And soon after his election last November, Mr Reagan set up a task force on strategic minerals chaired by Mr Daniel McMichael, president of the World Affairs Council of Pittsburgh, which had done much to legitimize the "resource war" concept.

Last June, for example, the World Affairs Council organized a conference in Pittsburgh on this theme, discussing in particular a report from Dr Daniel Fine, a resource analyst affiliated to the Massachusetts Institute of Technology's Mining and Mineral Resources Institute. Dr Fine argued that recent changes in Soviet minerals policy had conscious strategic implications; for example, that a decision to halt the export of titanium sponges to the United States "was based on an apparent decision to deter the US aerospace defence build-up forecast for the early 1980s".

The task force, which spurred the new Administration's general approach to minerals policy, also drew heavily on members of the Center on Economics and National Security, which had issued similar warnings of Soviet intentions in a report published last June. This committee, which receives support from the same conservative foundations as the Pittsburgh group, helped to draft a statement issued by the American Geological Institute last October warning the three main presidential candidates of Soviet efforts "to deprive this country of strategic minerals indispensable to its national defence".

Those who might be described as moderates on the strategic minerals issue accept its political importance. But they argue that major difficulties can probably be avoided by both renewed stockpiling and greater efforts to develop substitutes (which are beginning to go under the name "synterials"). Some suggest that, if there is going to be a "resource war", then it is more likely to be the result of competition with other customers, such as Japan.

The hard-liners push for a more aggressive stance. With the "national security" arguments playing a more dominant role, much of this push is now likely to come from within the Administration. One casualty of this thinking may be the United Nations Law of the Sea — the Reagan administration is concerned that the present UN proposals would not guarantee the United States access to sea-bed supplies of manganese and cobalt.

David Dickson

National Cancer Institute

Congress complains

Washington

Is Congress going sour on the conquest of cancer? This is the question raised in many minds by the tough questioning last week of the director of the National Cancer Institute (NCI), Dr Vincent T. DeVita, by the Senate Labor and Human Resources Committee. At one point, DeVita protested "I'm not a fool. I want this committee to be patting me on the head, not on other parts of my anatomy".

For the past decade, the committee has been one of the staunchest supporters of cancer research, helping to bid up the budget of NCI to more than \$1,000 million. Last week, however, the committee's hearing was delayed by an internal wrangle. Senator Edward Kennedy, senior minority member of the committee, complained that a dossier of complaints against NCI had been leaked in advance to the *New York Times* and the *Washington Post* without having been shown in advance to him and his Democratic colleagues. There was further dispute over the proposal by the committee chairman, Senator Orrin G. Hatch, that he should introduce each section of the questioning with a series of previously prepared questions. Even with the new low-power television lights, some senators perspired.

The chief bone of contention last week was NCI's award of a grant of \$910,000 to Dr Marc J. Strauss early in 1980 although Dr Strauss had resigned from his previous position at Boston University after allegations of falsified results two years earlier. A report on the allegations by the National Institutes of Health is expected later this summer, although the committee also had access to a report of the Food and Drug Administration which purports to document the mismanagement and even falsification of patient records as part of Boston University's work for the North-Eastern Cooperative Oncology Group.

The committee and Dr DeVita agreed last week on a version of the events at Boston University. Doubts about the adequacy of patient records arose within Boston University in June 1978, and Dr Strauss lost his job in August of that year. Since then data from the Boston studies have not been pooled with other data.

A year later, Dr Strauss was said to have applied for a further grant of \$910,000. Dr DeVita became director of NCI in January 1980, and agreed last week that he had deliberately decided not to inform NCI's advisory board of the allegations against Dr Strauss because the new application did not entail the treatment of human patients, because the case against him had not been proved and because Dr Strauss must be considered innocent until proved guilty.

The allegations said by Senator Hatch to have been justified by the Food and Drug Administration report include the use of

doses of ^3H -thymidine in excess of those required by the agreed protocols in the treatment of cancer patients; failure to obtain the informed consent of 112 patients to an experimental treatment; the treatment of 27 patients with ^3H -thymidine for breast cancer which was not one of the conditions specified in the protocol; and failure to keep complete X-ray records. Dr DeVita agreed that "all the allegations are curious and, if true, reprehensible".

The committee asked three broad questions. Why had the NIH investigation taken three years to bring nearly to completion? Why had Dr Strauss not been denied a further grant simply on the grounds that serious allegations had been made against him? And what would happen if the allegations were substantiated?

Dr DeVita admitted that "we were too slow off the mark" and also that "with the benefit of hindsight" it would have been wise to have told the advisory committee (a few weeks after becoming director of NCI) that Dr Strauss's Boston record was being investigated, even though that would probably have meant that the \$910,000 grant would not have been made. But he insisted that his discomfort before the committee was matched by his discomfort at "the idea of judging someone guilty until the investigation is complete".

Meanwhile, Dr DeVita said, procedures at NCI have been tightened up, with contracts reviewed by a central financially oriented staff. All contracts will in future be subject to peer review and he personally will take responsibility for seeing that funds are properly spent. If the allegations against Dr Strauss are proved, it seems there will still be time to recover \$600,000 of the 1980 grant.

US astronomy

Prospects for people

Washington

Anxiety about the future of astronomy in the United States is one of the recurrent themes in the final draft of the National Research Council report of the Astronomy Survey Committee. The committee, whose chairman is Dr George B. Field from the Harvard-Smithsonian Center for Astrophysics, has now submitted its fifth draft to the review procedures of the National Academy of Sciences.

The final draft is especially concerned about the plight of those astronomers who have been employed on fellowships or whose salaries have been charged to project grants for periods as long as six or eight years, and whose careers are now threatened by declining budgets. Astronomy differs from other fields of science in that there are no natural industrial outlets for academic astronomers, while universities are often unwilling as well as unable to employ such relatively senior people.

The draft report recommends a system of assistant professorships financed jointly by universities and the federal government. The acceptability of this proposal, for which there is no precedent in the United States, has yet to be determined.

The Field Committee also plans a series of recommendations of priorities for the future development of astronomy. Predictably it puts its weight behind the space telescope, but urges that support should also be given to second-generation instruments for the device. The launching of a γ -ray observatory (by NASA) and the building of the proposed 25-metre

millimetre-wave telescope are in the list of projects to be pursued, and there is a cautious welcome for the Princeton proposal to construct a gallium detector for solar neutrinos.

Further ahead, the Field Committee apparently wishes to give priority to an X-ray observatory even more advanced than Einstein and to a "new technology" telescope for optical and infrared observations. The proposal to deploy a 10-metre dish in an orbit about the Earth is put next on the list. The draft report has much to say about the need for technical and supporting services for the development of these projects.

Publication of the report is unlikely before October.

Uranium enrichment US price hike?

Washington

The question whether the United States should charge customers for enriched uranium more than the bare cost of enrichment surfaced again last week in the House of Representatives, this time because Congress is looking for ways of raising extra revenue so as to relieve the budgetary dilemmas forced on it by the Administration. But the chances of raising extra money quickly are small. And the Administration is clearly divided within itself about the merits of the proposal.

The charge for enrichment services at government enrichment plants has been a controversial issue since 1974, when it was planned that responsibility for non-military enrichment should be transferred to the private sector, and when everybody agreed that government prices should not undercut those of private manufacturers.

Last week, Congressman Richard L. Ottinger, chairman of the House Subcommittee on Energy Conservation and Power, opened a brief and hastily arranged enquiry on the subject with the blunt confession that the House Budget Committee needed to know this week whether the price of enriched uranium should be increased. One of his difficulties was that the Department of Energy, which argued for an increase of price in 1978, has now changed its mind. Dr William R. Voigt, who put the department's case for an increase three years ago, last week argued that the rules for enrichment pricing should not be changed simply so that Congress could spend more on other energy projects.

The General Accounting Office has been more consistent. In April, the director of the Office, Dr Dexter Peach, argued for prices that would take account of what private companies would have to set aside for state and federal taxes and for making a return on capital. His April calculation, repeated last week, was that the US enrichment business, now worth \$1,300 million a year, would yield an extra \$1,300 million

Vive la différence!

M. Pascal Clément would like French scientists to speak more French, *s'il vous plait*. In his report on the state of the French language, commissioned by the then President Giscard d'Estaing and presented recently to President Mitterrand, M. Clément — a parliamentarian and member of Giscard's Union pour la Démocratie Française — devotes a whole chapter to the invasion of English into science in France. French scientists are lazy about translating English technical terms, says Clément, preferring a kind of "Franglais"; and they even write their papers in English.

Something must be done, the report demands. Clément suggests that conferences conducted in English without translation facilities should get no support from state funds. This — if it ever took effect — would worry the Centre National de la Recherche Scientifique which supports much of the basic science in France; for two-thirds of

papers read at its conferences are read in English, while only one in five conferences have translators present.

Scientists have reacted to Clément's strictures with amused smiles. He is naive, they say. Much as they would like to speak and write in their native tongue, French scientists constitute only a tiny fraction of the world's scientists (7 per cent, estimates one). And any scientist worth his or her salt in France will be collaborating with groups abroad, with half of them probably American. And how many Americans can read or speak French?

Moreover, the inflexible attitude of people like Clément towards their language probably does more to help English penetrate French than hinder it, they say. For this attitude sees mistakes of foreigners attempting to speak French as *fautes* — faults — in the speaker, thus embarrassing some who would otherwise try to get by in the language.

As for the consequences of the report on French science, the thing is *une lettre morte* already.

Robert Walgate

between now and October 1986, one-third of that from overseas.

The nuclear industry (represented last week by the Edison Electric Institute) objects to the proposed increases of price partly on legal grounds — the amended Atomic Energy Act would have to be amended again — and partly on the grounds that a change in the price structure for enrichment services should be part of a more general review.

The costs of enrichment are already due to increase by 18 per cent from October. The proposed "fair pricing" increase would amount to a further 15 per cent, implying an increase of the cost of electricity generated by nuclear stations of between 1 and 2 per cent. Such an increase would not be decisive, which is why the decision on fair pricing may eventually turn on the competitiveness of US enrichment. Dr Peach quoted last week the latest prices charged by Eurodif as \$127 for a unit of separative work. For the first time, US enrichment is not the world's cheapest.

UK nuclear power

Missed again

The ailing nuclear construction industry in Britain, which suffered a ten-year gap in orders in the 1970s and over-committed itself to the advanced gas-cooled reactor, may now even be denied a quick entry to American pressurized water technology. For orders for key parts of the proposed Sizewell B nuclear power station — to be the first commercial pressurized water reactor (PWR) in the United Kingdom if it survives a public inquiry — may have to be placed abroad.

The reasons are cost and time. British industry is not tooled up to produce PWR components, in contrast with other companies — notably those in France, the United States and Germany — so costs will be higher and delays longer if orders are placed in the United Kingdom. Of the principal possible UK contractors, Babcock and Wilcox has indicated that it would need a government subsidy to compete with foreign suppliers; and Northern Engineering Industries has asked that foreign competition be ruled out.

Neither now seems likely. The "reference design" and safety case for the Sizewell reactor prepared by the National Nuclear Corporation is now with the Nuclear Installations Inspectorate for first scrutiny, and it includes a major extension of safety features compared with its American model, the Westinghouse reactor under construction at Callaway, Missouri. The independent emergency core cooling systems have been multiplied, the containment vessel extended to a double-wall construction and more concrete introduced into the reactor floor area to protect workers from the relatively high radiation levels of PWRs compared with the old Magnox stations.

French hiatus

The French Prime Minister, M. Pierre Mauroy, has confirmed a "freeze" on nuclear power construction in France, and promised a "great debate" in the National Assembly which will question the role and cost of nuclear power in France relative to that of coal and other sources of energy. But the idea of a national referendum on the matter has been pushed to the back of the stage, and the new government has raised no opposition to a major nuclear commitment made by the previous administration.

For in the last few days before the newly elected socialist president took over at the Elysée, the outgoing Prime Minister, M. Raymond Barre, agreed to the doubling of the capacity of the principal French oxide fuel reprocessing plant at Cap de la Hague near Cherbourg, run by the company Cogema.

Cap de la Hague has in recent years been dogged by malfunction and minor accidents. Its existing UP2 400 plant for oxide fuel (as used in pressurized water reactors) was designed to handle 200 tonnes a year; but in five years of operation it has been able to reprocess only 250 tonnes.

M. Barre's decree, if not overturned by the Mitterand government, will allow Cogema to spend 20,000 million francs (£2,000 million) on upgrading UP2 400 to UP2 800 (to handle 800 tonnes a year of spent oxide) and on building UP3, a completely new plant to handle an equal amount of spent fuel from overseas.

The technology of the new plant will be the same as before, but there will be parallel shearing and dissolution sections to give spare capacity if one part of the plant breaks down — small comfort perhaps for Cogema's six customers, Japan, West Germany, The Netherlands, Belgium, Switzerland and Sweden.

Some in France believe that the proposed national debate over nuclear matters could mean that the Cap de la Hague decision could be reopened, and that the decision to reprocess foreign fuel may be reexamined. **Robert Walgate**

Mr Brian George, who was recently appointed by the Central Electricity Generating Board to direct its interests in the PWR project, said on Monday that the board's approach to ordering for Sizewell might differ if it could clearly be seen to be part of a long programme of orders, for then it would be prudent to build up the UK nuclear industry. On the other hand, the first order might be the last. And Mr Reginald Flint, responsible for the reference design at the National Nuclear Corporation, confirmed that all orders for the PWR would have to be placed on a strictly commercial, competitive basis, with no preference for UK suppliers.

Robert Walgate

Yugoslavian nuclear power

Locals late reacting

The charging of Yugoslavia's first nuclear power station, at Krsko on the River Sava, has provoked a belated burst of opposition. During the construction of the reactor, a 664 MW Westinghouse unit, local feeling was muted. But when charging with uranium fuel was about to begin, the reactor became an issue in the Yugoslav press.

Krsko lies on the border between the republics of Slovenia and Croatia, which jointly built the power station. The commission in charge of reactor safety is likewise an inter-republic body, made up of scientists from the Josef Stefan Physics Institute in Ljubljana and the Rudjer Boskovic Physics Institute in Zagreb.

According to the Yugoslav news agency *Tanjug*, the commission decided in April this year that the trial operation of the station scheduled for July would have to be postponed. The commission is said to have found that measures to protect the environment, the staff and the nearby population had not been implemented, that there was no proper procedure for evacuating the staff in an emergency and no proper fire-fighting equipment. Nor had the problems of storing radioactive waste and treating the coolant water been properly dealt with.

The following day, however, *Tanjug* reported that Dr Maurice Rosen, director of the International Atomic Energy Agency's reactor safety department, had announced that there were no "technological obstacles" to the charging of the station. Nine days later, the Slovenian and Croatian authorities gave permission for charging to go ahead.

According to the Zagreb press, the Yugoslav safety committee tried to insist that certain safety work which Westinghouse and the power station management had planned for after charging, should be done first, but the recommendations were ignored. Significantly, *Vecernji list* of 2 June quotes Dr Marko Brankovic, head of the Croatian Commission for the Human Environment, as saying, "We cannot play around with nuclear reactors as if they were 'political factories'. They must be perfect in every technological respect, or else they must not start". A "political factory", in Yugoslav slang, is a factory erected for prestige reasons, without proper attention to technological details and which proves to be a waste of the usually considerable investment.

Part of the present trouble may be caused by local misunderstanding of the distinction between the charging of a reactor and its start-up. There is also considerable concern about the highly polluted state of the Sava, from Krsko down to its confluence with the Danube. Rumour (downstream in Zagreb) at-

tributes the state of the river to the construction of the power station; in fact, the chief offender is a paper-mill which also happens to be sited at Krsko.

Although naturally concerned with safety and environmental questions, the members of the safety committee remain optimistic that the power station will, in the end, be completed, though probably not by the deadline of October/November.

Meanwhile both sides of the belated nuclear debate can find comfort in the recent news that after years of prospecting, a rich uranium deposit (Yugoslavia's second) has been found near Probistip in Macedonia, while in the middle of May a federal Council for Solar Energy was established in Belgrade.

Vera Rich

London University

More trouble ahead

Some of the smaller colleges of London University may have to close and others amalgamate, but the large colleges with international reputations in many subjects should be kept largely intact. That is the gist of suggestions put forward in the second discussion document of the committee on the reorganization of London University, issued last week.

The problems facing the university have grown since the committee issued its first document last December. Now the university has to contend with an 8 per cent cut in its grant over the next three years in addition to the loss of income from the expected shortfall in overseas students.

The University Grants Committee is expected to tell each university how much money it has for 1981-82 and to allocate student quotas for 1983-84 at the beginning of July. But the London University committee urges that the implications of the expected cut in income are so severe and the timescale so short that discussion of what should be done must start now.

The document says that the university must cater for at least a 10 per cent decrease in the number of students and should reduce the number of buildings it occupies. The suggestion most likely to raise hackles is that Chelsea College, which caters mainly for science and mathematics, be drastically slimmed down or even closed. Chelsea has a good reputation in science education, nursing and the history and philosophy of science, says the committee. But it is not confident that the college's other departments are as good. The good should be sorted out from the bad by peer review, says the committee. Departments surviving the review may be sufficient to form a small college on one site — Chelsea is now widely scattered over several sites. Otherwise they should be incorporated into another college.

The sites that should be kept intact are those supporting colleges with high reputations in a wide range of subjects and, if

consistent, those with low costs. Imperial, University and King's colleges and the London School of Economics are likely to emerge largely intact. They may even gain from closer cooperation with some of the smaller colleges — for example, the committee would like to see Queen Elizabeth College, a small but successful institute of science, increase its links with Imperial College. Poor departments in Bedford and Westfield colleges should be weeded out and the two combined on the Bedford site, with the help of money acquired by the sale of Westfield's valuable Hampstead property.

Judy Redfearn

Research in Poland

Cutting red tape

The Polish production ministries have proved ineffective in coordinating research and development and their role should be phased out, according to Dr Janusz Gorski, Minister of Science, Higher Education and Technology. Writing recently in the weekly *Polityka*, he argued for decentralization and a restoration of decision-making power to the universities and academic institutions.

Academic autonomy, in research as in every other field, has been widely urged in Poland during the last few months by the Academy of Sciences, by the universities, and by the Solidarity "circles" in research and academic institutes. Indeed, Dr Gorski's article was an answer to a swingeing attack, also published in *Polityka*, by Maciej Ilowiecki, one of Poland's leading science writers. According to Ilowiecki, only the restoration of academic autonomy to the scientists could cure the long list of ills of Polish science, including an "absurd" system of financing.

Dr Gorski's article, for the first time, makes some concrete suggestions for improvements. In particular Dr Gorski criticizes the "hierarchy of problems", which has been the basis of Polish science planning since the early 1970s.

According to this scheme, all research funding, with the exception of a small basic research sector, is divided between various "problems" associated with the needs of the national economy. These are classified, in order of importance, as "governmental", "key", "interdepartmental", "departmental" and "branch" problems. Research topics are normally subdivided between a number of institutes and research teams, whose work is co-ordinated, according to the class of problem concerned, by the Academy of Sciences, and/or the relevant ministries.

Not surprisingly, this scheme has produced a mushrooming bureaucracy, and a scramble among research teams to get their own project as high up the ladder as possible. Now, however, Dr Gorski has proposed eliminating the bottom two levels, "departmental" and "branch",

Psychiatrist on trial

Dr Anatolii Koryagin, consultant psychiatrist to the Moscow "Working Group to Investigate the Use of Psychiatry for Political Purposes", went on trial in Moscow last week. Until his arrest last February, Dr Koryagin was the last member of the group still at liberty in Moscow — his fellow psychiatrist, Dr Aleksandr Voloshanovich, emigrated in February 1980 and the remaining members of the group are either in prison or labour camp.

Dr Koryagin practised psychiatry in Soviet hospitals for 17 years. Since the working group was set up in 1977, he has examined a number of patients consigned to psychiatric clinics as a result of their conflict with the Soviet State system, and found no sign of disorder.

Although the Soviet authorities have recently abandoned the confinement on psychiatric grounds of well-known dissidents, according to Dr Koryagin, the practice has become widespread for citizens of no great standing who commit some act construed as anti-Soviet. In an article which he sent to *The Lancet* shortly before his arrest, and which was published in April, Dr Koryagin analysed a number of these cases. In many of the cases he cited, the "patients" were simply confined for some weeks or months and then released without receiving any treatment — proof positive, he concludes, that the reason for containing them was isolation, not therapy.

Dr Koryagin was charged with "anti-Soviet agitation" — a far more serious offence than that of slandering the Soviet state, with which several of his fellow members of the commission were charged. In an appeal to foreign colleagues, which reached the West a few days before his trial, Dr Koryagin stated that the "transformation of psychiatry into an instrument of politics" was an attempt "drastically to distort the nature of this humane science". While praising the efforts of Western psychiatrists to combat these abuses of their profession, he urged them to be even more decisive in the future.

precisely where the role of the production ministries is greatest. In their place, he suggested a system of direct contracts between industry and research institutes, possibly based on competitive tenders.

Whether Dr Gorski's ministry will survive to implement any changes, however, is in doubt. Proposals on rationalizing government spending may lead to the ministry's responsibilities going to enlarged education and industry ministries. In any case some feel that the much-needed reforms cannot be settled while the new legislation on higher education and new statutes for the Academy of Sciences are still only in the discussion stage.

Vera Rich

CORRESPONDENCE

Post Office inertia

SIR — It is interesting that a scientific journal, and one as influential as *Nature*, should take up the question of the telecommunications monopoly "deregulation" (*Nature* 26 March, p.279, 23 April, p.619). The importance of this matter is not generally appreciated, and your articles contain some very pertinent comments.

This issue transcends party politics; though no doubt the fate of the British Telecommunications Bill will be influenced by the usual "state ownership versus free enterprise" dogma. The fact is that a country possessing an efficient inexpensive telecommunications system operates with an advantage over one which possesses a relatively expensive inefficient one.

Implementation policy for a universal communications system is important; it would be hard to argue that a remote farmhouse in Cumberland should be without a telephone because resources have been diverted to the profitable business area. But the wealth-producing capacity of the nation mainly resides within manufacturing and service industries, whose ability to create that wealth is substantially affected by the availability of efficient communication services.

It appears that US deregulation policy, falteringly followed since 1968, has not resulted in "cream-skimming" at the expense of services in remote areas. There is an extraordinary range of facilities available in that country which are unavailable here.

I have a list of 60 organizations in the United States offering a range of telephone and data communications equipment; there are probably many more. The number of companies in this country catering for this demand, excluding US subsidiaries, can be counted on the fingers of one hand. The reason is twofold; first, the demand for these devices here is small as yet. Second, the inertia of large bureaucracies encourages a safe policy and the purchase of very long-lasting equipment. The UK telecommunications industry has until very recently been asked to supply out of date equipment to the Post Office. The industry's concentration on the manufacture of this equipment left it in a weak position when tendering for the large international telecommunications contracts which have been placed during the last few years. They were awarded elsewhere.

CITECH, A.E. CAWKELL
Uxbridge, Mddx, UK

Bovine units

SIR — With reference to Dr John Damuth's letter "Population density and body size in mammals" (*Nature* 23 April, p.699), it has been a general "rule-of-thumb" of the US cattle industry that the annual sustained "carrying capacity" of grazing land is approximately equal to one cow unit per section (square mile) per inch of annual rainfall, with one additional inch of rainfall, with one additional inch of rainfall being assumed for each 1,000 feet of altitude over 5,000 feet; and, a cow unit being defined as a "mother-cow with calf to natural weaning, plus one bull for about each 30 mother-cows."

Seattle, Washington, USA — C.P. ANDERSON

Look harder

SIR — Andrew Brooks in his letter (*Nature* 7 May, p.7) raises a problem which is of wide relevance for the effective application of research and development effort. He is correct in believing that the lack of training in use of information results in much unintentional duplication or misdirection of research. It is particularly galling to us that the area of astronomy in which Mr Brooks presumably undertook his research is covered by the "computerized" *Physics Abstracts* which forms part of the INSPEC database. This database covering physics, electrotechnology and computing is in common with other databases accessible worldwide through a variety of on-line services.

The major difficulty is to make potential users aware of the range of information services and to train them in their use. Inclusion of such training in all undergraduate courses would obviously be a solution, although time for this training is not easily found in heavily-loaded courses. We in INSPEC would be willing to cooperate with universities and colleges to supplement their own efforts at either the undergraduate or postgraduate level.

PETER CLAGUE
INSPEC, Institution of Electrical Engineers,
Hitchin, Herts, UK

SIR — We read with great interest Andrew Brooks' letter (*Nature* 7 May, p.7) concerning the problems of literature searching as part of the PhD research project. For some years we have been tackling this problem at Leicester Polytechnic, where user education is now particularly well-developed. A team of five Academic Librarians has been appointed to train all levels of students in the importance of information and in the techniques involved in its acquisition, storage and retrieval.

We are particularly aware of the daunting size of the task facing postgraduate students in science and technology, when searching the literature as part of their own research. With this in mind we offer courses specifically dealing with the problem at this level, tailored to each postgraduate's own research area.

By means of lectures, demonstrations, practical work and individual tutorials the researchers are introduced to the diverse range of published and unpublished sources in their subject field. The principles and techniques involved in both manual and computerized information searching are explained, and demonstrations are given to show the potentialities of accessing information via on-line bibliographic data-bases. The library offers computerized information retrieval facilities to all research students, many of whom find that a combination of manual and computerized searching provides the best solution. Guidance is also given in related areas such as thesis presentation, report writing techniques and coping with foreign language material.

Similar facilities are provided in several other academic institutions. More widespread encouragement of this trend would go a long way towards solving the problems outlined by Mr Brooks.

RUTH WEBB
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The fact is . . .

SIR — Disputes among the supporters of evolution are often only "a matter of presentation". For example, "Evolution is a fact" is a statement I thoroughly dislike, but is understandable to those who have traced the many meanings of "fact". The postulates of a well established theory are sometimes called facts. Mitchell¹ gives the postulates of the chemiosmotic theory and then points out that they are now considered to be facts by most persons. So the postulate-fact confusion arises from the failure to specify one's temporal point of view and adherence to certain views.

The terms "true", "proof", "belief" and "falsification" are often carelessly used when discussing evolution. The first two terms are so final in their meaning that they have mostly disappeared from the discussions of the complex theories. Theories of very limited scope can often be proven true, but the broad, general theories never are. Since "belief" is a favourite term of the creationists and since for them it is something strong and final, scientists could well omit the term, as many have.

Only when one examines the structure of knowledge in evolution can one evaluate "falsification" used in its simple, literal sense. Evolution consists of a system of hundreds of theories² which stem from Darwin's two major theories — the kinematic theory of descent with modification and the dynamic theory of natural selection. These theories in Darwinian and neo-Darwinian forms have spawned and are spawning hierarchies of subtheories. Some of the postulates of the major theories and subtheories and some of the subtheories as a whole may be falsifiable, but usually only new postulates and new subtheories are falsified and discarded. The usual fate of established postulates and theories is not falsification but limitation. Decades ago Delbruck³ cautioned biologists about the folly of discarding good laws and theories on the grounds that they had limited ranges of applicability.

The testing of a major theory rests with the testing of its subtheories and the fate of the major theory is determined not by a single test, but by an evaluation of the successes and failures in its system of subtheories⁴⁻⁶. "Falsification" is not then of much use in evaluating the major theories of evolution. The notion that evolution is not scientific as it is not falsifiable is based on a thin understanding of the structure of major theories, the way they develop and the part being tested.

Since creationists are planning the "ultimate court battle" in which they "will try to establish the 'religious' nature of evolutionary beliefs"⁷, it is essential that the scientific community clarify and solidify its views and terminology. We must find and state clearly our fundamental agreements so that our differences in presentation cannot be misconstrued and distorted.

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NEWS AND VIEWS

Overfishing: is there a solution?

from A.J. Southward

THE late Michael Graham, sometime Director of United Kingdom Fishery Research, always maintained that "fisheries that are unlimited become unprofitable . . . or inefficient"¹. Nevertheless, argument continues over the extent to which observed changes in fish populations are the result of overfishing or of natural causes, including climatic change²⁻⁴.

A good example of such disagreement can be seen in the recent history of the formerly profitable herring fishery in the southern North Sea. Some scientists believed that the decline after 1955 was in part a natural change, others that it was due to overfishing of the adult shoals, and yet others that the industrial fishery for juveniles was responsible⁵. As a result of the failure to agree about the causes, conservation measures were delayed, and the fishery collapsed. Even today some scientists are unconvinced that fishing can reduce stocks to below the point of recovery and cause changes in the whole ecosystem. For this reason two recent communications to *Nature* are significant: one pointing towards the causes of a severe decline in certain species (K. Brander, vol.290, p.48), the other showing massive changes in the ecosystem that may have resulted from removal of predators by overfishing (K. Sherman *et al.*, this issue, p.486).

In the case of the common skate, *Raia batis*, Brander has developed the classic method of comparing 'recruitment' — essentially the sum of breeding intensity and survival of the young up to the size when they are taken by fishing gear — with the subsequent mortality each year afterwards due to natural causes and fishing. Skate lay only a few large eggs each year, and although these are well protected by a horny case, the young that hatch from them are at once large enough to be captured by the meshes of standard trawls. Skate also take a long time to reach maturity — as much as 11 years. Thus even a moderate level of fishing is enough to lead to virtual extinction of the population. This explains the almost total disappearance of skate from the Irish Sea during the past 20 years, and may explain a much earlier decline in the western English Channel⁶. Other species of *Raia* are nearly as vulnerable as skate to a moderate level of fishing, and another elasmobranch fish

that produces only a few young per year, the spur dog, *Squalus acanthias*, may be equally endangered⁷. This species virtually disappeared from the western Channel between 1920 and 1950⁸, and more recently has declined in the northern North Sea⁹.

In contrast to the elasmobranchs, most teleost fish produce immense numbers of eggs, and it used to be argued that the effects of fishing on these species must be very small compared with the great natural mortality during the early stages of their life history¹⁰. This argument was probably not true even a hundred years ago¹, and today's technology, especially the use of sophisticated sonar to locate the shoals and large purse-seines to trap them, can produce fishing mortalities up to ten times greater than the natural mortalities¹¹. Removal of a species on a scale like this must surely influence the rest of the ecosystem, for example by allowing increases in abundance of a competing species or of organisms that were eaten by the fish. Some scientists believe this happened in the 1970s in the northern North Sea, where, after the decline of herring and mackerel, there were increased catches of 'trash' species such as small gadoids, sand eels and sprat^{12,13}. This 'gadoid outburst' has also been attributed to natural causes, such as an improved 'match' between the spawning season of the fish and peak production of the plankton on which the young feed in the spring, possibly related to a trend in climate^{2,14}.

The detection of a similar outburst across the Atlantic by Sherman and colleagues is therefore significant. These workers report increases in abundance of young sand eels, during the past 6 years, over a wide area of sea off the north-east coast of the United States, from George's Bank to Cape Hatteras. Although there has been variation between years in the position of maximum abundance, all three major fishing regions surveyed show increases of more than an order of magnitude in the number of sand eels. The stocks of herring and mackerel in the same regions appear to have declined greatly since 1972, and it is difficult to avoid the conclusion that removal of some of its major predators has allowed the 'opportunistic' sand eel to increase.

However, this increase might also be attributed to natural changes. Good correlations can be shown between sea-temperature trends and fluctuations of several commercial fishes in the Gulf of Maine and off Nova Scotia^{15,16}. Similar correlations can be shown for UK waters, but in each case the variations in catches are small and are correlated with quite small changes in sea temperature.

With the gadoid outburst in the North Sea, and the increase in sand eels in the North-West Atlantic, we are faced with much greater changes in abundance. A comparable change in the ecosystem, with differences of one or two orders of magnitude in the numbers of the young of demersal fish, took place in the western Channel: first a decrease when herring gave way to pilchard as the dominant pelagic fish, then an increase when the pilchard declined and mackerel moved in¹⁷. This change in the English Channel has always been accepted as natural², probably related to the effect of climatic fluctuation on the position of a boundary between different ecosystems¹⁷. The events in the North Sea and the North-West Atlantic are difficult to relate to changes in climate, because they have occurred over a wide range of latitude and there is little sign of shifts in geographical distribution of key species or of organisms from other habitats, such as were reported from the English Channel^{4,17}. It is therefore simpler to accept, for the moment, the hypothesis that the increases in 'trash' species are due to removal of herring and mackerel. Such an hypothesis should be easy to test, by

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stopping or reducing the fishing of the herring or mackerel, whereupon the numbers of 'trash' species should fall; but whether effective international action can be achieved to sustain such a test is another matter.

No 'easy' solution is available for the elasmobranch fishes, which are endangered by such a low level of fishing that only a complete ban on all fishing over

a wide area would allow recovery. As Brander says, we have to face the prospect that skate, and similarly sensitive species, will be fished out (or become extinct) as a consequence of the exploitation of other fish. □

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stagger between adjacent triple helical molecules in the collagen fibre. These occur at a distance, D , of 234 amino acids, or 78 triplets. He suggests that if gene duplication has occurred, the repeated domain is likely to be of length D , or a simple fraction of D . More recently, Hofmann, Fietzek and Kuhn¹⁴, analysing the $\alpha 1(I)$, $\alpha 2(I)$ and $\alpha 1(III)$ chains, also found the D repeat, but in addition found smaller repeats of $D/3$, $D/6$, $D/11$ and, perhaps more significantly, $D/13$ (18 amino acids). Also emerging from these data is the fact that the homologies within one repeat are more conserved across species than from one repeat to another within a chain. The most likely explanation for this is early intrachain duplication with subsequent duplication of the whole chain.

Recently, the application of recombinant DNA technology to the analysis of the collagen $\alpha 2(I)$ gene, and the subsequent determination of DNA sequences, have yielded more definitive predictions of the size of the hypothetical primordial collagen gene. The isolation and characterization of genomic clones covering approximately half the sheep $\alpha 2(I)$ ^{15,16} and the whole of the chick $\alpha 2(I)$ gene^{17,18,5,6} have revealed that this gene is extremely large and has the greatest number of intervening sequences of any gene studied to date⁹. Five kilobases (kb) of coding sequence are distributed over at least 38 kb of DNA containing 49 introns varying in size from less than 100 base pairs (bp) to more than 3,000 bp. Introns are largest and exons smallest within the region coding the triple helical portion of the polypeptide. More strikingly, DNA sequence analysis of some of the exons within this portion of the chicken gene reveal that these exons always end at a position corresponding to the Y position of the Gly-X-Y triplet and never in the middle of a triplet^{5,6}.

Yamada and colleagues⁵ have sequenced eight exons of the chick $\alpha 2(I)$ gene and find that seven of these are 54 bp (18 amino acids) long, while the eighth is 99 (54 + 45) bp. Five additional exons have now been sequenced by the same group — three of 54 bp and two of 108 (2 × 54) bp (B. de Crombrughe, personal communication). These authors therefore propose that the first collagen gene arose by amplification of a single coding unit of 54 bp. They predict that all collagen genes, whether in sponges, nematodes, or man, will be found to have exons of this length. In addition, this small primordial gene may have functioned in the primitive cell, and small collagenous regions, such as those found in Clq and acetylcholinesterase, could be remnants of such a function. The occurrence of an exon of 99 bp can be explained by various recombinational and

Collagen evolution

from Ellen Solomon and Kathryn S.E. Cheah

COLLAGEN has a long evolutionary history, and has been found in all invertebrate and vertebrate phyla studied, including such primitive invertebrates as fresh-water sponges and sea anemone. Considerable advances have been made in the understanding of the biochemistry of collagen, and its precursor procollagen, with respect to molecular structure, assembly and post-translational processing¹⁻³. Until recently, however, attempts to analyse the genetic origins of this protein have had to rely on amino acid sequence data which, because of the size, complexity and difficulty of isolation of the collagen molecules, have been difficult to obtain. Recombinant DNA technology and DNA sequencing methods now provide an alternative means of analysing the origins of the collagen genes. During the past year the structure of one such gene, $\alpha 2(I)$, has been determined in some detail, and this in turn has provided a substantial clue as to its evolution^{4,5}.

Basic to all collagenous proteins is the amino acid tripeptide Gly-X-Y, which is repeated approximately 330 times in the most abundant and best studied, Type I collagen, about 1,600 times in annelid cuticle collagen⁶, and approximately 29 times in the chains of Clq⁷. These molecules are all triple helical structures, comprised either of three identical chains, or two different chains, in the ratio of 2:1. Recently a new dimension has been added to the biology of connective tissues with the discovery that, in addition to being a very ancient molecule, collagen is also exceedingly complicated genetically: it exists, at least in vertebrates, in at least five distinct types, for which a minimum of nine genes must exist to code for their constituent chains^{8,9}. If proteins with collagenous domains such as Clq⁷ and acetylcholinesterase¹⁰ are added to this list, the number of genes in the family increases to at least 15 or 16.

The evolution of these genes is interesting from several points of view. Because of the periodic nature of the Gly-X-Y repeat in these proteins, and the general conservation of sequences, it has been assumed that the collagen genes are all related evolutionarily through duplication

and divergence of an ancient collagen gene. However, although the fundamental unit which expanded was clearly the Gly-X-Y tripeptide, the question has been raised as to whether there was an initial expansion of this triplet to a small primordial gene. This small gene could then have duplicated to give a long chain, which itself duplicated and diverged, or could have been the basis for the independent generation of several long chains.

Attempts to answer such questions and to evaluate the evolutionary relationships between the collagen chains have relied on statistical analysis of amino acid sequence data. Most of the available data are for the $\alpha 1(I)$ and $\alpha 2(I)$ chains, with some data for $\alpha 1(II)$ and $\alpha 1(III)$ ³. As much of the sequence data is incomplete and could be non-representative of the entire chain, the conclusions about evolutionary distances are quite provisional. Nonetheless, calculations¹¹ indicate that the $\alpha 1(I)$ and $\alpha 2(I)$ chains diverged about 7×10^8 years ago, about the time of the separation of vertebrates and invertebrates. The $\alpha 1(I)$ chain has evolved slowly, at about the rate of cytochrome *c*, while the $\alpha 2(I)$ chain has evolved more quickly, at about the rate of haemoglobin α and β . On the basis of sequence comparisons of four α chains, an evolutionary tree has been proposed, in which successive gene duplications resulted in separate genes coding for $\alpha 1(III)$, $\alpha 2(I)$, $\alpha 1(II)$ and $\alpha 1(I)$ ¹², in that order. Although some of these calculations may be provisional, the data clearly show that the conservation of sequence of each of the chains is far greater for the same chain across species (variation in about 5–10 per cent of non-glycine residues) than it is between different chains within a species (variation in about 30–60 per cent of non-glycine residues).

A model of a primordial collagen gene which duplicates to form one chain would predict that internal homologies would be found within that chain, provided, of course, that the sequence were sufficiently conserved for these to be detected. McLachlan¹³, using a comparison matrix on the amino acid sequence of the $\alpha 1(I)$ chain, has detected significant regularities occurring at a distance related to the

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mutational events, all of which must involve the deletion of 9 bp (corresponding to one Gly-X-Y triplet). Yamada and co-workers predict that for any collagen gene for which the protein helicity is conserved, all insertions or deletions in the helical coding regions will be 9 bp or multiples of this length.

Wozney and colleagues⁶ have arrived at a similar conclusion based on the analysis of seven exons derived also from the chick $\alpha 2(I)$ gene. Additional data from this laboratory bring the total number of exons which they have sequenced to fourteen (H. Boedtker, personal communication) of which seven are 54 bp, two are 108, two are 45 and three are 99. To accommodate the exons of 45 and 99 bp, these authors suggest that, during evolution, duplication of the 54-bp gene was accompanied by additions and deletions of 9 bp.

One of the exons studied by Wozney *et al.* contained the coding sequences covering 45 bp at the end of the triple helical region of the pro $\alpha 2(I)$ chain as well as 204 bp of the adjacent non-triple helical carboxy-terminal domain. Included in this non-triple helical region is the procollagen peptidase cleavage site. This is somewhat surprising since according to the concept of introns separating functional domains, one might have expected to find an intron separating sequences specifying helical and globular regions of the procollagen molecule, and perhaps also at the peptidase cleavage site. Any hypothesis involving the duplication of a 54-bp primordial collagen sequence to give rise to a procollagen gene must therefore also explain how this sort of exon could have arisen. Loss of introns is the most likely possibility.

Although the hypothesis of evolution of the collagen genes by duplication of a small exon of 54 bp is attractive, it must be remembered that so far it is based solely on the $\alpha 2(I)$ gene with the majority of exons not yet analysed. Data on some of the other genes are eagerly awaited. □

Polymers by synchrotron radiation

from Paul Calvert

SMALL-ANGLE neutron scattering has made a dramatic impact in polymer physics over the past few years. It is therefore interesting to speculate whether anything similar might come from synchrotron radiation, especially in view of the fact that the synchrotron radiation source (SRS) at the SRC Daresbury Laboratory was commissioned last June.

In brief, the SRS provides an intense, continuous source of radiation from the hard X-ray region out to microwaves. The radiation is predominantly polarized and pulsed in about 120-ps pulses at 2-ns intervals. What can actually be done at Daresbury will depend on the installation of suitable monochromators, cameras and detectors. In the X-ray region the available intensities are three to five orders of magnitude greater than from conventional rotating anode sources, permitting exposures of a few seconds in wide- and small-angle diffraction so that time-dependent structural changes can readily be studied. In the UV, visible and IR, the intensities available can be equalled by arc lamps or lasers though the continuous spectrum of the SRS may be an advantage. In the long-wavelength range, synchrotron radiation again becomes more intense than conventional sources.

Three reports published recently on the use of synchrotron radiation for X-ray studies of polymers all concentrate on the measurement of changes during phase transformations. Forgacs (Budapest) together with co-workers at Novosibirsk report the use of the Vepp-3 storage ring at the Institute of Nuclear Physics in Novosibirsk to study melting crystallization and drawing in polyethylene and polypropylene (*J. Polymer Sci.* **18**; 2155, 1980). A position-sensitive counter was used to record the scattering from 7 to 35°, 2 θ , into 1,000 channels. The authors claim to get recognizable curves within 1 second but generally used counting times of 30–60 seconds. They present data on high- and low-density polyethylene and polypropylene during heating from room temperature through the melting range. Time dependence is not reported, possibly because none was seen. The time taken for the sample to reach thermal equilibrium at each temperature was several minutes which may exceed the time required for changes in the structure. Crystallization of polypropylene could be followed as a function of time at 120°C although again the process was half over before thermal equilibrium was reached. They were also able to follow the relaxation of a sample after rapid uniaxial stretching.

Elsner, Zachmann and Milch used the DORIS storage ring at DESY in Hamburg to study crystallization of oriented

polyethylene terephthalate on heating from the glassy state (*Makromolec. Chemie* **182**; 657, 1981). The complete small-angle X-ray pattern was recorded over an area of diameter 4 cm using an X-ray-sensitive vidicon tube. Exposure time was 10 seconds. The long period, which is a measure of crystal thickness, was found to decrease during crystallization, in agreement with previous studies made on partly crystallized and quenched samples. Also at DESY, Koch and co-workers (*Polymer Bull.* **1**; 709, 1979) measured crystallization in stretched polyisobutylene rubber. Samples were extended in 0.5 second and data then collected over a series of intervals of 5–30 seconds. The crystallization was shown to follow the Avrami equation with an exponent of one. When the sample was allowed to retract the crystallinity disappeared within 5 seconds.

Shorter exposures than this have been achieved with conventional sources. Using a rotating anode tube Stein and co-workers (*J. Polymer Sci.* **46**; 15, 1974) were able to obtain rather fuzzy diffraction patterns from polyethylene in 1/60th second and observed the development of a single peak in stretched polyethylene with a resolution of about 0.025 second. Clearly the synchrotron source has the potential to improve greatly on this if the sample can be stretched rapidly enough. Very little is known about the melting kinetics of polymers and dynamic X-ray studies using the SRS could be very useful, but this requires sample holders capable of reaching thermal equilibrium in a few seconds. On the other hand, a suitable choice of samples and conditions will give crystallization rates which can be easily followed. However, because the process is intrinsically heterogeneous, we really need to know what happens in a very small volume of material as crystals grow through it; this means a beam size of the order of 1 μm .

These applications only skim the surface of the potential uses of the SRS (for a review, see *Synchrotron Radiation Research* eds H. Winick and S. Doniach, Plenum, 1980). Extended X-ray absorption fine structure (EXAFS) has proved very informative about metal ions in enzymes and has great promise for the study of surfaces and of catalysts. X-ray topography and soft X-ray microscopy using the SRS also look interesting. Synchrotron radiation is a very powerful spectroscopic tool; the challenge is to think of how to use it. □

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Atom-specific bond breakage

from Michael L. Knotek

A recent series of discoveries in the field of electron- and photon-stimulated desorption (ESD and PSD) promise to deepen understanding of surface and molecular chemistry and the interaction of ionizing radiation with matter. Those studying ESD and PSD have long recognized that a low-energy ionizing particle (an electron or photon with $0 < \text{energy} < 10^4$ eV) causes bond dissociation by electronic excitation^{1,2}. Now it has been found that bonds to a specific surface atom can be broken by exciting an inner shell or 'core' electron on that atom³⁻⁸. Furthermore, on an ordered surface the emitted (desorbed) ions are seen to emerge in well defined beams which reflect the geometry of the bonding configuration^{7,9}. Both of these features are due to highly localized energy deposition and bond breakage phenomena³. By using a simple electron source or the intense, tunable light available from a synchrotron^{4,6,7,10}, the electronic and geometric structure of specific species bonded to specific surface atoms can be studied. The techniques and concepts derived in studying the surface bond and its dissociation by ionizing radiation will be widely applicable to a variety of important chemical systems.

Stimulated desorption experiments have been used to characterize some of the fundamental properties of a bond by studying its dissociation with ionizing radiation. In a typical experiment the energy of an ionizing particle is increased until threshold for desorption of ions is noted. Significantly, the desorption threshold of a given species coincides with the excitation of one of its own core electrons, or a core electron of the atom to which it is bonded³⁻⁸, as was first demonstrated for the desorption of positive oxygen ions (O^+) from TiO_2 . The O^+ desorbed when a 'core-hole' was created (that is, a core-level ionized) on the Ti site to which it was bonded³. It was subsequently demonstrated that the core-hole decays by having a valence or bonding electron fall into the vacant core-hole. One or more valence electrons will also be ejected to carry off the energy released in the decay (so-called Auger electrons). For an ionic solid the decay of a core-hole on a metal atom (cation) can remove enough charge from its neighbouring anion to cause the latter to change from a negative to a positive ion and hence be in an extremely repulsive state from which it will desorb as a positive ion (Fig. 1). The mechanism is not as simple for covalently bonded solids, but the removal of two or more electrons from

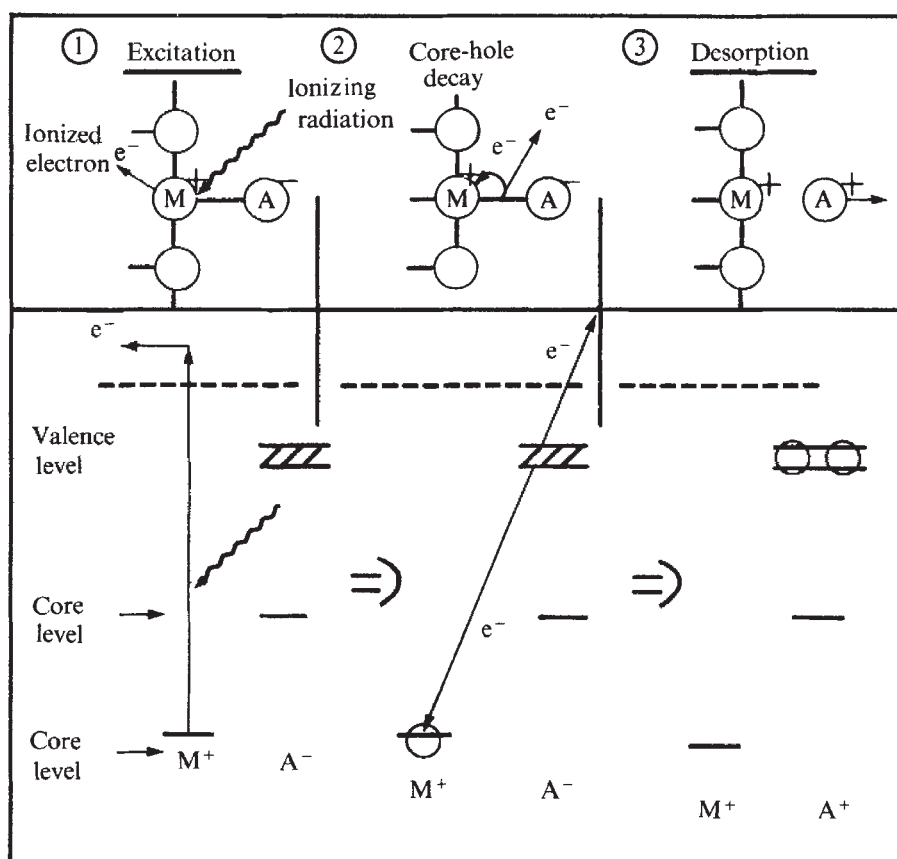


Fig. 1 Ions are emitted from surfaces (or bonds broken in a molecule or solid) in a three-step process. In an example of ionic bonding ($\text{M}^+ \text{A}^-$): 1, Ionizing radiation excites an electron from a core level in a bonding site atom (M). 2, The resulting 'core-hole' decays by absorbing a valence electron and emitting one (or two) more. 3, The resulting multiple valence hole final state is highly repulsive and the resultant adsorbate, (A^+), desorbs.

the bond can often cause it to break with the emission of ions — a so-called Coulomb explosion⁵.

Two crucial features of this kind of bond dissociation are that the excitation of a core-hole stores an enormous amount of highly localized energy in an atom and that the decay of this hole produces multiple valence-hole states localized on the order of atomic dimensions. The concept of intrinsic localization of the Auger final state was explored for a range of chemical systems in relation to the Auger process and now to desorption and dissociation¹¹⁻¹⁵. Two or more valence holes created on an atom or in a specific bond strongly repel each other. Contrary to simple intuition, the more strongly the holes repel one another, the longer they are locked together^{11,12}. Since this unstable configuration cannot be relieved through electronic processes in a time that is short compared with the characteristic desorption time, localized bond breakage occurs when the valence holes are properly arranged and the electronic state has a suf-

ficient lifetime.

These results clearly demonstrate that the bonding site for a given chemical species can be determined by measuring the desorption threshold and matching that energy with tabulated core-level energies of

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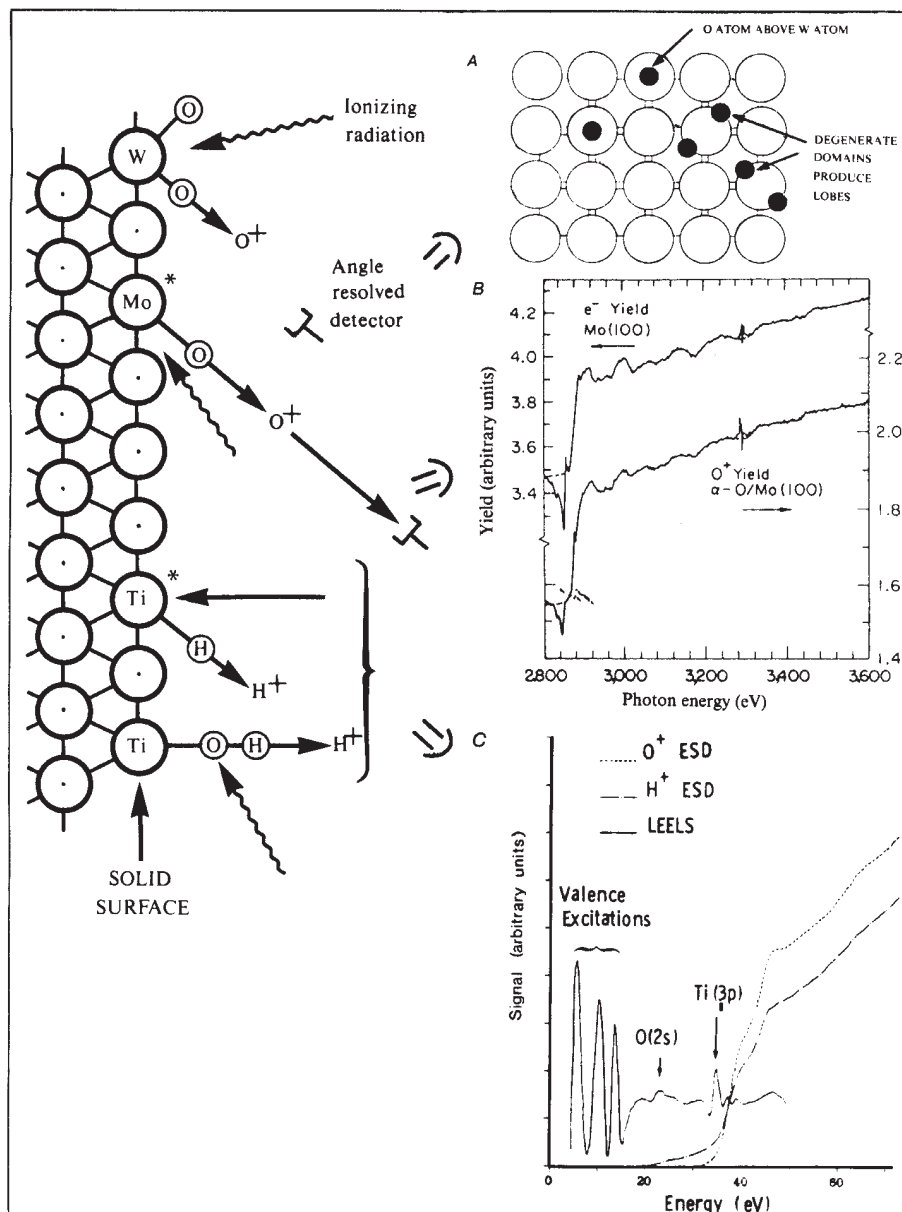


Fig. 2 Excitation of specific surface atoms breaks bonds only to those atoms. The ions are emitted in well defined beams which can be observed by imaging devices to give directly an image from which the bonding structure as in *A* can be derived. Structure observed in X-ray-excited desorption can be analysed to determine the geometric structure in the vicinity of the excited atom (*B*). For an adsorbate such as C-O on a surface, excitation of the carbon (1s) level gives both C⁺ and O⁺ in desorption while excitation of the oxygen (1s) level gives O⁺ (*C*). If a given species (say hydrogen) is bonded to more than one kind of surface atom, then threshold for desorption will be observed at both bonding site atom core level ionization potentials as in the example of hydrogen on TiO₂.

the elements. Each atom has a distinct set of core-level energies which makes the task straightforward and unambiguous. Since apparently only first nearest-neighbour bonds are broken, this technique can determine the bonding sites of all the desorbing species. One problem, however, is that some species do not desorb, desorb weakly, or desorb only as neutrals.

Even more fruitful is the study of the behaviour of the ion yield of a particular species as a function of energy above the excitation threshold. These thresholds are not simply abrupt changes in yield, but contain complex spectral structure within roughly 20–30 eV of the edge (so-called

near edge structure) and extending hundreds of electron volts above the edge (extended fine structure). These structures arise because the desorption of ions will simply be proportional to the cross-section for core-hole creation on the adsorption site or adsorbate atom. The spectrum reflects the X-ray absorption spectrum of the excited atom and yields a detailed picture of its electronic and geometric environment. This specificity allows examination of a surface with a variety of adsorbates and bonding sites by extracting the excitation spectrum of each site/adsorbate combination independently of the others.

The correlation of core-hole excitation thresholds with stimulated desorption yield thresholds has been made for metals, semiconductors and insulators^{3–10}. The extraction of information from the structure in the desorption yield spectrum is in an early stage of development, but has been successfully applied to a PSD Surface Extended X-ray Absorption Fine Structure (PSD/SEXAFS) study of oxygen on molybdenum¹⁰. The radial distribution function (r.d.f) of the Mo surface atoms originally bonded to desorbed O⁺ ions was determined from modulations in the yield spectrum above the Mo (2s) absorption edge. The bonding site molybdenum r.d.f. (basically a measure of the Mo–Mo distance) was found to remain essentially unchanged upon adsorption of oxygen on the surface — a rather surprising result. The desorbed oxygen apparently comes from MoO₃-like species formed during oxidation. SEXAFS structures were also seen in the desorption of ions from BeO, Al₂O₃ and SiO₂ surfaces⁸.

Distinct beams of ions in stimulated desorption were first observed in ESD experiments⁹ (ESDIAD for ESD Ion Angular Distributions), and more recently in PSD⁷. ESDIAD patterns reflect the site symmetry and bond angles of adsorbed species on several metal surfaces, and are also sensitive to surface details such as adsorption at step sites, and thermal vibrations of the adsorbate. Significantly, these measurements define the local geometry of the surface–adsorbate bond in a statistical sample of the surface and do not require long-range order to determine structure. ESD/PSD thus has the unique capability to determine structure both from angle-resolved techniques and from analysis of spectral features. The former gives the adsorbate structure (bond angle and symmetry) while the latter characterizes the environment of either the adsorbate or bonding site atom.

Although research has been confined to systems of traditional interest to surface scientists, these studies can easily be extended to more complex structures including organometallics — it may be possible to probe the central metal atom in porphyrins, or perhaps cell membranes where the environment of a specific metal atom can be monitored. This experimental approach is extremely sensitive to hydrogen (which desorbs most readily of all species) and to the details of the electronic and structural nature of hydrogen bonding; so its application to problems involving hydrogen chemistry will undoubtedly be important.

New concepts of desorption and dissociation are being explored and will reveal new features of desorption phenomena and deepen understanding of surface chemical processes. Probably the most important result of this research is a new perspective for surface bonding. This will lead to new ideas not only regarding surfaces, but also radiation-induced chemistry and damage. □

Neuropeptides: do they integrate body and brain?

from Susan D. Iversen

DE WIED AND BOHUS were the first to demonstrate that an extract of the posterior pituitary delayed the extinction of active shock avoidance behaviour in the rat¹. Subsequent experiments indicated that the active substance was vasopressin, and synthetic Lys⁸-vasopressin given in a single dose (3.0 µg) systemically caused a long-lasting delay in extinction². These results have led to the hypothesis that vasopressin modulates processes underlying memory consolidation. If this claim were to be substantiated, vasopressin might have useful therapeutic effects in alleviating memory disorders in man, such as those seen in Korsakoff's psychosis and Alzheimer's disease.

An important question concerns the site at which vasopressin acts to produce its effects on memory. A potent analogue, Arg⁸-vasopressin, has a significant effect on extinction behaviour when administered intraventricularly in doses as low as 0.025 ng. When the dose-response data are compared for the subcutaneous and intracerebral routes of administration, it is clear that much smaller doses are effective when injected directly into brain³. Thus, de Wied and his colleagues favour a central nervous system (CNS) site of action. Furthermore, using immunohistochemical staining techniques it has been demonstrated that vasopressin-containing neurones of the magnocellular hypothalamic nuclei project widely to innervate various autonomic centres in the brain stem and spinal cord, and vasopressin fibres are also found in limbic septum⁴. The enhanced memory performance after local injections of arginine vasopressin (AVP) into these limbic areas, has been correlated with changes in CNS noradrenaline function⁵. A number of intriguing facts support the hypothesis that an interaction between vasopressin and limbic forebrain noradrenaline is important for cognitive functions related to memory. Studies on human brain have recently revealed that the locus coeruleus

contains the highest level of vasopressin outside the hypothalamus⁶. The locus coeruleus is the origin of the noradrenaline innervation of hippocampus, and lesions to this system result in learning impairments in animals when trained responses must be modified⁷. Degeneration of the locus coeruleus has been observed in the brains of patients dying with Alzheimer's disease⁸ and degeneration of the dorsal noradrenaline pathway has been implicated in Korsakoff's disease⁹, an amnesic syndrome said to be improved by vasopressin treatment¹⁰.

The question of a central site of action of AVP is raised again in this issue of *Nature* (p.491). Le Moal and colleagues have confirmed the effects of systemic AVP on extinction behaviour in the rat, and report that a novel synthetic antagonist of AVP can abolish this behavioural effect. Within the same dose range, they also demonstrate an effect of AVP on blood pressure, which was reversed by the antagonist at doses effective against the behavioural response. The authors suggest that there is a causal relationship between the effect of AVP on blood pressure and the prolongation of extinction behaviour, and that the visceral arousal induced by AVP and reflected in the blood pressure change induces, via visceral afferents to the CNS, cerebral arousal "sufficient to alert the rat and to cause the perseverance of ongoing or escape behaviours".

They suggest that the reported effects of AVP on brain may also be due to peripherally triggered activation of visceral integrative centres. In the experiments where AVP has been injected into the ventricles or limbic sites, it is possible that the circumventricular organ systems may be the site of action rather than structures within the brain itself. It seems premature to reject one or other of these hypotheses. For example, the vasopressin analogue Desglycinamide lysine vasopressin, which is practically devoid of classical endocrine activity, has an effect on extinction of avoidance behaviour¹¹. It would seem that a presser response to vasopressin is sufficient but not necessary for the effect on behaviour.

A more important implication of the paper is that vasopressin and other neuropeptides may act at both peripheral and central nervous system sites in an integrated fashion. Vasopressin released in brain would appear to act on the noradrenergic pathway arising in locus coeruleus. It is interesting that visceral afferent information reaches brain stem areas (including locus coeruleus) rich in vasopressin innervation. In certain behavioural situations visceral and central arousal, induced independently by the release of

vasopressin systemically and in brain, may synergize to achieve coordinated physiological and behavioural response. A parallel example is to be found in the studies of cholecystokinin¹² and bombesin¹³ both of which have been reported, after systemic injection, to reduce food intake in the fasted animal. Traditionally such effects are thought to involve satiety centres of the CNS, which modulate ingestive behaviour. However, Deutch¹⁴ and his colleagues found that these peptides induce profound physiological changes in gut and suggest that the resulting malaise or strong visceral arousal is sufficient to account for the behavioural effects of these peptides. In support of this view, Deutch has reported that subcutaneously administered cholecystokinin or bombesin provides a sufficient visceral cue for conditioned taste aversion to develop.

Again, CNS activation, secondary to visceral arousal, may largely account for the behavioural effect of a peptide. The importance of the present article by Le Moal *et al.* is that it forces us to re-evaluate the peripheral effects of peptides in accounting for their effects on behaviour. In the past decade it has been exciting to discover that a large number of peptides, with established endocrine and physiological functions, exist in brain. Perhaps we have too readily assumed that their behavioural effects reflect direct actions on the CNS. □



100 years ago

Mr. Josceline Bagot and Mr. Drummond, of the Grenadier Guards, accompanied by Mr. T. Wright, the winner of the International Balloon Contest, went up in a balloon from the Crystal Palace on the 1st inst. at 1 p.m. When the ropes were loosed they ascended to the height of 5000 feet, and travelled slowly in a south-westerly direction for the distance of about eight miles. The balloon then suddenly sank, but ballast being thrown out, it rose again to 8000 feet, and traversed in the direction of Epsom. The aeronauts then descended in a field about a quarter of a mile from the Grand Stand, which they reached in time to witness the race for the Derby.

An opera performed on the stage of the Paris Grand Opera House was heard satisfactorily at the Rue Riche Opera House by a number of French officials a few days ago. The feat was performed with the new Ader telephone, of which the peculiarities have not been made public. The performance will be repeated at the International Exhibition.

from *Nature* 24, 9 June, 132, 1881

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REVIEW ARTICLE

The traditional and a new version of the mouse *H-2* complex

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The H-2 complex has traditionally been interpreted as a maze of regions, subregions and loci coding for different traits. The two main theses presented here are, first, that a single H-2 locus is pleiomorphic in that it controls several functions such as allograft rejection, cell-mediated lymphocytotoxicity, mixed lymphocyte reaction, immune response, immune suppression and restriction of T-cell specificity; and second, that the physiological function of the H-2 complex is to guide T lymphocytes in their function of distinguishing self from non-self, and that all other H-2-controlled traits are artificial derivatives of this basic function. These two theses lead to a new, simplified interpretation of the H-2 complex.

ACCORDING to the traditional view, the major histocompatibility complex (MHC) is a chromosomal segment bounded by two loci originally chosen because their products are responsible for rapid rejection of foreign tissue grafts and are easily detected by serological methods^{1,2}. Each locus in this segment is then considered part of the MHC, whether it is functionally related to the bounding loci or not. The organization of the *H-2* complex, the mouse MHC, is shown in Table 1. The complex is divided into four regions, *K*, *I*, *S* and *D*, and the *I* region is further divided into five subregions: *I-A*, *I-B*, *I-J*, *I-E* and *I-C* (the so-called *G* region, originally placed between *S* and *D*, has recently been demonstrated to be inseparable from the *S* region^{3,4}). Each region or subregion controls several traits, and each trait is presumably controlled by a separate locus. The relationship between the various loci in each region or subregion is unknown but in no case have genetic recombinants been obtained by separating the individual loci. A brief description of the *H-2*-controlled traits is given below (see also Table 1).

H-2-controlled traits

Rejection of allografts. A piece of tissue or an organ taken from one mouse and transplanted to another mouse with a different *H-2* complex is destroyed by an immune process called the allograft reaction. The alloantigens which trigger this reaction are controlled by histocompatibility (*H*) loci which map in the *K*, *I-A*, *I-E* and *D* regions⁵.

Cell-mediated lympholysis (CML). Thymus-derived (T) lymphocytes of one mouse can be induced by cells of another mouse with a different *H-2* complex to transform into cytolytic (killer) cells that can lyse appropriate target cells. This lysis is usually measured by the release of cytoplasmically bound isotopes such as ⁵¹Cr. The loci controlling the CML-inducing alloantigens map in the same regions as the *H* loci and the two are usually considered to be identical.

Mixed lymphocyte reaction (MLR). Resting T lymphocytes of one mouse can be stimulated to proliferate by mixing them in tissue culture with lymphoid cells from another mouse with a different *H-2* complex. The proliferation can be measured by the incorporation of ³H-thymidine into the DNA-synthesizing cells. The lymphocyte-activating determinants responsible for the MLR are controlled by *Lad* loci which map predominantly in the *I-A* and *I-E* subregions⁶, although weak *Lad* loci have also been placed in the *K*, *I-J*, *I-C* and *D* regions⁷⁻⁹.

Control of immune response. There are striking differences in the ability of different mouse strains to produce antibodies to a great variety of antigens¹⁰. Some strains are high responders which produce high levels of antibodies to a given antigen, others are low responders or nonresponders which produce low levels of or no antibodies to this antigen. The variation in the immune response ability is controlled by immune response (*Ir*) loci, many of which map in the *I* region of the *H-2* complex¹¹ (however, *Ir* loci mapping in the *K* region have also been reported^{12,13}). In some cases, the nonresponder can be turned into a high responder by immunization with an antigen attached to another molecule (for example, methylated bovine serum albumin, MBSA¹⁴). In such situations, preimmunization with the antigen alone often suppresses the response to the subsequent challenge with the complexed antigen, a response that, without the pretreatment, is high¹⁵. The suppression is thought to be mediated by special immune suppression (*Is*) genes that map in the *I-A* and *I-C* subregions. Immune suppression is generally mediated by a subpopulation of T lymphocytes, the suppressor T cells (*T_s*), some of which carry a cell-surface alloantigen controlled by the *I-J* subregion¹⁶. The *I-J* antigens are detected by specific alloantibodies which abolish immune suppression when incorporated into some of the immune suppressive systems, presumably by blocking or killing the suppressor T cells.

Control of T-B collaboration. Although antibodies are produced by descendants of B lymphocytes, the differentiation of B cells into antibody-producing cells is triggered by the interaction of B lymphocytes with the helper T lymphocytes (*T_h*). This interaction is mediated by hypothetical 'interaction structures' controlled by *I*-region loci¹⁷. Originally, T cell-B cell compatibility of interaction structures was thought to be required for successful collaboration¹⁸, but this conclusion was later challenged¹⁹.

Restriction of T-cell specificity. Virus-infected cells stimulate the differentiation of precursor T cells into cytolytic T (*T_c*) lymphocytes capable of destroying infected target cells. The T-cell response to virus infection shows dual specificity: the stimulated cells are specific not only for the virus but also for *H-2* molecules borne by the inducing cells and controlled by the *K* and *D* regions²⁰. As a result the mature *T_c* lymphocytes lyse only target cells infected with the same virus and bearing the same *K* or *D* molecules as the stimulating cells. Similar *H-2* restriction of T-cell activity has also been described for the *T_h* lymphocytes. To help B cells, the *T_h* cells must first be activated by an antigen presented to them by a macrophage-like (antigen-presenting) cell. The *T_h* cells also show dual specificity in that

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Table 1 The traditional version of the *H-2* complex

Region	<i>K</i>		<i>I</i>		<i>S</i>		<i>D</i>	
Subregion	<i>I-A</i>		<i>I-J</i>					
Molecular product	<i>A_α, A_β, A_ε</i>	<i>E_α</i>	<i>I-B</i>	<i>I-E</i>	<i>I-C</i>	<i>SS, Slp</i>	<i>D, L</i>	
Trait (locus)	<i>K</i>	<i>E_α</i>	<i>I-B</i>	<i>I-E</i>	<i>I-C</i>	<i>SS, Slp</i>	<i>D, L</i>	
Serologically detectable antigens (<i>H-2, Ia, SS, Slp</i>)	++++	++++	—	+	++	—	++++	++++
Rejection of allografts (<i>H</i>)	++++	+++	—	—	++	?	—	++++
Cell-mediated lympholysis (<i>H</i>)	++++	+++	—	—	+++	?	—	++++
Mixed lymphocyte reaction (<i>Lad</i>)	++	++++	—	+	++	+	—	++
Control of immune response (<i>Ir</i>)	+	++++	++	—	+++	—	—	—
(<i>Is</i>)	—	++++	—	—	—	++++	—	—
<i>T_h</i> -cell marker	—	—	—	++++	—	—	—	—
Control of T-B cell collaboration	—	++++	?	?	+++	?	—	—
Restriction of <i>T_h</i> -cell specificity	++++	—	—	—	—	—	—	++++
Restriction of <i>T_h</i> -cell specificity	—	++++	?	?	+++	?	—	—
Control of complement activity	—	—	—	—	—	—	++++	—

they recognize not only the antigen but also *H-2*-encoded molecules of the antigen-presenting cell. However, in contrast to the *T_c* cells, the molecules recognized by the *T_h* cells are controlled by loci in the *I* region²¹.

Control of complement activity. Complement is a system of interacting serum proteins (mostly enzymes), activated in a coordinated fashion, which participate in immunological functions such as lysis of bacteria and macrophage chemotaxis. Complement-encoding loci have been mapped in the *S* region of the *H-2* complex²².

Gene products

The usual way of detecting *H-2*-controlled molecules is to cross-immunize strains differing only in the *H-2* complex (*H-2* congenic lines) or only in part of it (*H-2* recombinant strains), and to produce antibodies against these molecules. These antibodies can then be analysed by any of the known serological methods and individual *H-2* determinants can thus be defined. Serologically detectable molecules controlled by all *H-2* regions or subregions except *I-B* and *I-C* have now been identified. The determinants borne by the *K* and *D* molecules are referred to as *H-2* antigens, those controlled by the *I* subregion are termed *I* region-associated, or *Ia* antigens.

The *K* region codes for at least one polypeptide chain with a molecular weight (MW) of 45,000 (ref. 23), which is non-covalently associated with the small polypeptide β_2 -microglobulin encoded by a locus on chromosome 2 (ref. 24) (the *H-2* complex is located on chromosome 17). The *D* region controls at least two polypeptide chains, *D* and *L*, each of MW 45,000 and each associated with β_2 -microglobulin polypeptide chains.

The *I* region codes for a minimum of four polypeptide chains *A_α*, *A_β*, *A_ε* (*E_β*) and *E_α*; the α chains have a molecular weight of ~32,000, the β chains of ~28,000²⁵. The chains are synthesized separately in the cytoplasm but combine to form heterodimers before they integrate into the plasma membrane: the *A_α* combines with *A_β* to form an *A* molecule and the *A_ε* (so designated because the encoding locus maps in the *I-A* subregion) combines with *E_α* to form an *E* molecule. Some inbred strains carry a mutation affecting the *E_α*-encoding locus which results in the absence of *E_α* chains in the cytoplasm; strains carrying this mutation do not express the *E* molecule on the cell surface, as in the absence of the α chain, the β chains cannot integrate into the membrane²⁶. The products of the *I-B*, *I-J* and *I-C* subregions have not been identified.

The *S* region codes for two molecules, *Ss* and *Slp*, both of which correspond structurally to the C4 component of the complement cascade but only one of which (*Ss*) has been demonstrated to have C4 activity in the functional test²⁷.

This traditional interpretation of the *H-2* complex grew out of total ignorance of the physiological function of the *H-2* loci. It is understandable that in a situation where seemingly unrelated traits were discovered to be *H-2* controlled, the complex seemed to immunologists to be a maze of separate loci, regions and subregions, the interrelationships of which remained unclear. However, now that we have some idea about

the *H-2* function (see below), we should ask whether the complex has not been over-interpreted and whether it cannot be viewed in a simpler way. Here we propose a new interpretation of the *H-2* complex which is simpler and unifies the loci. For some, this article may merely be a formulation of what they have thought for some time; for others it may represent a radical departure from their own concepts; for us, it is the only interpretation that makes sense.

Unification of *H-2* loci

One of the main theses of this article is that a single *H-2* locus controls a variety of traits—in other words, that the loci are pleiomorphic. There is much data supporting this idea, of which we will cite here only a few selected examples.

Unity of loci in the *K* and *D* regions. These two regions contain loci which govern rapid allograft rejection, strong CML, weak MLR, immune response to a few antigens and specificity of cytolytic T lymphocytes^{1,2}. We propose that all these functions are carried out by the serologically detectable, 45,000-MW molecules controlled by the *H-2K* and *H-2D* (*H-2L*) loci in these two regions. The main evidence for this conclusion comes from the study of *H-2* mutations; over 24 mutations mapping in the *K* (most of them) or the *D* (a few) region have been described²⁸. All have been demonstrated to affect the *H-2K*, *H-2D* or *H-2L* and no other loci in the genome. Several are known to have resulted in amino acid substitutions in the serologically detectable, 45,000-MW polypeptide chain²⁹. Functional tests have revealed that a single mutation in the *H-2K*, *H-2D* and *H-2L* locus can generate new antigenic determinants which are detectable by serological methods, lead to graft rejection, stimulate CML and MLR, change the immune response pattern and affect the specificity of cytolytic T lymphocytes directed against virus-infected cells²⁸. There is, therefore, every reason to believe that all these traits are the function of a single molecule—the 45,000-MW, serologically detectable molecule—and there is thus no need to postulate separate loci for the individual traits.

Unity of loci in the *I* region. We shall deal first with the *I-A* and *I-E* subregions—the two subregions known to control serologically detectable *Ia* antigens. All the *I-A*- or *I-E*-associated functions are carried out by the *A* and the *E* molecules and we shall now discuss the evidence for the identity of the individual loci.

Identity of *H* and *Ia* loci. A unique opportunity to test the relationship of the *H* loci governing graft rejection *in vivo* or CML *in vitro*, and the *Ia* loci has been provided by the discovery that some mouse strains carry a nonexpressor allele at the *E_α* locus which prevents the appearance of *E* molecules on the cell surface²⁶. We have exploited the existence of nonexpressor *E_α* alleles (which we designate *E_α⁰*; the expressor alleles are designated *E_α⁺*) in the following way^{30,31}. We selected from the collection of available mouse strains four pairs in which the members of each pair were genetically identical except for a short segment of the *I* region, including the *I-E* subregion. Furthermore, the strain combinations were such that in each

pair one member carried the E_{α}^0 and the other member the E_{α}^+ allele. When we then attempted to generate primary CML with cells derived from these strains, we succeeded in the following combinations: B10.A(4R) anti-B10.A(2R), B10.D2(R107) anti-B10.A(3R), B10.S(7R) anti-B10.S(9R) and D2.GD anti-B10.D2(R101). All attempts to generate CML in the reciprocal direction (such as B10.A(2R) anti-B10.A(4R), B10.A(3R) anti-B10.D2(R107)) failed. Examination of the genetic constitution of the eight strains used (Table 2) reveals that positive CML reaction occurred in combinations in which the responder strain carried the E_{α}^0 allele (and hence did not express the E molecule) and the stimulator the E_{α}^+ allele (and hence expressed the E molecule); no CML could be generated against cells that lacked the serologically detectable I-E molecule. This correlation between generation of CML and the presence of the E molecule on stimulator cells can hardly be a coincidence; most likely the serologically detectable I-E molecule is the one that generates the CML and is thus the product of the *I*-region *H* gene. A similar case can also be made for the *H* locus mapping in the *I*-A subregion³². Here the CML reaction directed against a product of this subregion can be blocked by Ia-specific antibodies³³, and a mutation affecting the serologically detectable Ia antigens also affects the *H* locus³⁴. The combined data thus best fit the interpretation that the *H* and *Ia* loci in the *I*-A and *I*-E subregions are identical.

Identity of *Lad* and *Ia* loci. The evidence for equating the *Lad* loci, which govern the MLR, with the *Ia* loci, responsible for the serologically detectable determinants, is of a similar nature to that for the *H* and *Ia* loci: the reaction can be inhibited by anti-Ia sera³⁵ and the same mutant mentioned above also affects MLR³⁴. In addition, the *Lad* and *Ia* determinants are known to have the same tissue distribution, being particularly abundant on B lymphocytes and macrophages, or macrophage-like cells, and scarce on most other cells³⁶.

There are two additional, rather peripheral pieces of evidence which nevertheless argue strongly in favour of our thesis. The first concerns an older observation³⁷ that in strain combination B10.A(2R) and B10.A(4R), MLR occurs only in one direction (B10.A(4R) anti-B10.A(2R)). This unidirectionality, which has long puzzled immunologists, can now be explained simply by the fact that the two strains differ only in the *I*-region segment around the *I*-E subregion and that the B10.A(2R) strain expresses the E molecule whereas the B10.A(4R) strain does not. The occurrence of MLR thus again correlates with the expression of serologically detectable E molecules on the surface of the stimulating cells.

The second piece of evidence has been provided by Fathmann *et al.*³⁸ who observed that T lymphocytes of strain A (haplotype *H*-2^a with alleles *K*^k, *I*-A^k, *I*-B^k, *I*-J^k, *I*-E^k, *I*-C^d, *S*^d and *D*^d), when sensitized to (A × C57BL/6)_F₁ cells respond on restimulation more strongly to the *F*₁ than to the C57BL/6 cells (haplotype *H*-2^b with alleles *K*^b, *I*-A^b, *I*-B^b, *I*-J^b, *I*-E^b, *I*-C^b, *S*^b and *D*^b). The authors explain this result by postulating the existence of "hybrid determinants" in the *H*-2 heterozygous

cells, but a simpler explanation is possible. In the A anti-(A × C57BL/6)_F₁ combination, two kinds of cell are activated: those which recognize A_B determinants (in combination with A_α^b or A_α^k) and those recognizing E_B determinants (in combinations with E_α^k). When tested against the C57BL/6 parent which carries the null allele at E_α and hence does not express cell-surface E_B, only the anti-A_B cells (not the anti-E_B cells) can react. For this reason the response against the *F*₁ hybrid is stronger than that against C57BL/6 cells. This observation again strongly argues that the lymphocyte-activating determinants are carried by the same molecules that bear the serologically detectable Ia antigenic determinants.

Identity of *Ir* (*I*s) and *Ia* loci. While the only way to study *Ir* genes was to immunize mice and test their sera for the presence of specific antibodies, the question of the *Ir*-gene product was almost unanswerable. Fortunately, however, an assay is now available in which part or the entire immune response process occurs in tissue culture and is thus amenable to cellular and molecular manipulation. This 'T-cell proliferation assay'³⁹ consists of immunizing mice with a given antigen, collecting immune T cells several days after the immunization, and culturing these together with macrophages and the same antigen as that used for immunization. The macrophages presumably present the antigen to the T cells which then respond by proliferation and this can be measured by the incorporation of ³H-thymidine. Numerous tests done using this assay have invariably proved that the degree of T-cell proliferation *in vitro* correlates well with the level of antibody production to the particular antigen *in vivo*: high responders defined by the antibody assay are also high responders *in vitro* and T cells from strains that produce low levels of or no antibodies *in vivo* proliferate poorly or not at all *in vitro*⁴⁰. This correlation is not surprising in view of the fact that helper T cells are necessary to initiate B-lymphocyte differentiation, leading to antibody-producing cells.

We used the T-cell proliferation assay to test the relationship between the *Ir* and *Ia* genes in the following way⁴¹. Three antigens were selected, the response to which is known to be under *Ir* control: synthetic polypeptide of randomly assembled amino acids glutamic acid and alanine (GA), another synthetic polypeptide of the randomly assembled amino acids glutamic acid, lysine and tyrosine (GLT), and a natural protein lactate dehydrogenase B (LDH_B). The antibody response to GA is known to be controlled by an *Ir* gene that maps in the *I*-A subregion⁴². We have tested the GA response of several strains in the T-cell proliferation assay and found complete agreement with the *in vivo* data: in this assay the response is also controlled by the *I*-A subregion. We then repeated the experiments but added to the culture monoclonal antibodies specific for Ia-bearing molecules. We found that the proliferation of T cells derived from high-responder strains was inhibited by the Ia-specific antibodies but only by those reacting with the A molecule of the strain from which the cells in the culture were derived; antibodies specific for the E molecule of the same strain or those specific for the H-2K and H-2D molecules had no effect on the reaction. This experiment and similar ones done by others⁴³⁻⁴⁵ provides evidence that the Ia-bearing molecules are involved in an important way in the control of the immune response to antigens. Even more revealing are the data obtained with the second antigen. The antibody response to GLT has been reported to be controlled by two *Ir* genes⁴⁶, one mapping in the *I*-A and the other in the *I*-E subregion. In addition, the response to this antigen shows a phenomenon known as *Ir*-gene complementation⁴⁷. It has been observed that some of the recombinants derived by crossing of two nonresponder strains are high responders. This has been explained by assuming that two genes are involved in the response and that neither of these genes alone can effect high responsiveness but that the combined action (complementation) of the two genes does result in high responsiveness.

We tested the response to GLT in the T-cell proliferation assay and again confirmed the mapping data obtained by *in vivo*

Table 2 *H*-2 genotypes of recombinant strains used in the study of phenotype-genotype relationships

Strain	<i>H</i> -2 haplotype	Alleles at <i>H</i> -2 loci					
		<i>K</i>	<i>A_B</i>	<i>A_α</i>	<i>E_B</i>	<i>E_α</i>	<i>D</i>
B10.D2(R101)	<i>g1</i>	<i>d</i>	<i>d</i>	<i>d</i>	<i>d</i>	<i>b</i>	<i>b</i>
B10.GD	<i>g2</i>	<i>d</i>	<i>d</i>	<i>d</i>	(<i>d/b</i>)	(<i>b</i>)	<i>b</i>
B10.A(2R)	<i>h2</i>	<i>k</i>	<i>k</i>	<i>k</i>	<i>k</i>	<i>k</i>	<i>b</i>
B10.A(4R)	<i>h4</i>	<i>k</i>	<i>k</i>	<i>k</i>	(<i>k</i>)	(<i>b</i>)	<i>b</i>
B10.A(3R)	<i>i3</i>	<i>b</i>	<i>b</i>	<i>b</i>	<i>b</i>	<i>k</i>	<i>d</i>
B10.D2(R107)	<i>i7</i>	<i>b</i>	<i>b</i>	<i>b</i>	(<i>b</i>)	(<i>b</i>)	<i>d</i>
B10.S(7R)	<i>i2</i>	<i>s</i>	<i>s</i>	<i>s</i>	(<i>s</i>)	(<i>s</i>)	<i>d</i>
B10.S(9R)	<i>i4</i>	<i>s</i>	<i>s</i>	<i>s</i>	<i>s</i>	<i>k</i>	<i>d</i>

The strains are listed in pairs where members of each pair differ only at the E-encoding loci. Alleles in parentheses are not expressed on the cell surface. The loci are listed according to the simplified interpretation of the *H*-2 complex given in the text.

antibody testing: there are indeed two genes involved, mapping respectively in the *I-A* and *I-E* subregions⁴¹. However, when we tried to inhibit the proliferation with Ia-specific monoclonal antibodies, we discovered that such inhibition was possible only with the E molecule-specific and not with the A molecule-specific antibodies. We explain these data as follows. In contrast to GA, the immune response to GLT is controlled by the E molecule, more specifically by the gene coding for the E_β chain, which is located in the *I-A* subregion. However, because the control is dependent on the expression of the E_β chain on the cell surface and as this depends on the presence of the E_α^+ gene, which maps in the *I-E* subregion, at the phenotype level the response to GLT seems to be controlled by two genes, one in the *I-A*, the other in the *I-E* subregion. In some recombinants derived from two nonresponder strains, a high responder E_β allele of one parent, which was not phenotypically expressed in the parent because it was combined with an E_α^0 allele, combines with an E_α^+ gene, becomes expressed, and results in responsiveness.

The combined data on GA and GLT responsiveness indicate that *Ir*-gene effects can be fully explained by assuming that the control of immune responsiveness occurs through the serologically detectable A or E molecules, and thus that the Ia-bearing molecules are the long-sought products of the *Ir* genes. The response to some antigens (for example, GA) is controlled by the A molecule, whereas that to other antigens (for example, GLT) is controlled by the E molecule. This conclusion is supported by the study of responsiveness to LDH_B, which also reveals additional complexity and which we shall discuss later.

We propose that the *Is* genes represent situations in which two H-2 molecules become involved in the response: one controls the generation of helper T cells and the other of suppressor T cells. The latter then act on the former and suppress the response. A precedent for this type of control exists in the response to LDH_B (see below). The proposition can be tested experimentally using monoclonal Ia- or H-2-specific antibodies.

Identity of loci controlling T_h-cell specificity with Ia loci. This genetic relationship follows from our experiments and others⁴³⁻⁴⁵ in which the T_h-cell function was blocked by Ia-specific antibodies. The attachment of antibodies to the Ia-bearing molecules on antigen-presenting cells presumably prevents T_h cells from recognizing these molecules and therefore from being stimulated by the antigen. Furthermore, as the genetic restrictions of T-B cell collaboration *in vivo* correlate perfectly with those determined by the T-cell proliferation assay *in vitro*, the postulated interaction structures¹⁷ are nothing but the Ia-bearing molecules.

In summary, all the different functions attributed to the *I-A* and *I-E* subregions of the *I* region have a common denominator in the serologically detectable, Ia determinant-bearing molecules. It is these molecules that are the products of the loci controlling these functions and hence the loci encoding the different traits are all identical.

Problematic subregions of the *I* region

We shall discuss the remaining three *I* subregions separately as they each present a different problem in their interpretation. **I-B subregion.** This chromosomal segment was originally

defined in the manner shown in Fig. 1. Here, the $H-2^a/H-2^b$ heterozygote gave rise to two reciprocal haplotypes, $H-2^{15}$ and $H-2^{h4}$. The $H-2^a$ haplotype confers low responsiveness to antigens such as LDH_B or mouse IgG2a myeloma protein; the $H-2^b$ and both recombinant $H-2$ haplotypes confer high responsiveness to these antigens^{48,49}. This observation was interpreted as indicating the existence of a new *I-B* subregion separated from the *I-A* subregion by the recombinational event leading to the $H-2^{h4}$ haplotype and from the *I-J* subregion by crossing-over resulting in the $H-2^{15}$ haplotype (see Fig. 1). The control of immune responsiveness to the LDH_B and IgG2a, as well as a few other antigens, has remained the only known function of the *I-B* subregion: this subregion does not affect MLR or CML, does not influence graft rejection, and does not control any serologically detectable product.

We have tested again the responsiveness to LDH_B and to the IgG2a myeloma protein using the *in vitro* T-cell proliferation assay and found that the $H-2^a$ is a nonresponder haplotype and that the $H-2^b$, $H-2^{h4}$ and $H-2^{15}$ are high responder haplotypes just as in the antibody assay⁵⁰. However, by using the antibody-inhibition method we found that the responsiveness of the $H-2^b$ haplotype could be completely abolished by the addition to the culture of monoclonal antibodies specific for the A molecule. This observation suggests that the generation of T_h cells in response to LDH_B or IgG2a is controlled by the *I-A* rather than by the *I-B* subregion. This discrepancy between formal genetic analysis and functional testing was eliminated when we performed similar experiments with the $H-2^a$ cells. The addition of E molecule-specific antibodies to the culture of $H-2^a$ cells, which are normally nonresponders, transformed them into high responders. Instead of blocking, the antibodies in this case enhanced the response. As we obtained a similar conversion of nonresponders into high responders by the removal from the culture of suppressor T cells, we explained the data as follows. In mice expressing the A and the E molecules, the response to LDH_B and IgG2a is controlled by both these products but the E molecule controls the generation of suppressor T cells which then inhibit helper T cells, the generation of which is controlled by the A molecule. The coating of the E molecule with specific antibodies prevents the recognition of these molecules and thus the induction of suppression; the proliferation of T_h cells is then unopposed. In strains that do not express cell-surface E molecules (for example, the $H-2^b$ strains) only T_h cells are generated and the mice then show a high responder phenotype. This interpretation completely disposes of the *I-B* subregion as the response to LDH_B and similar antigens is fully explained by the interaction of cells recognizing *I-A*- and *I-E*-controlled molecules. We suggest that the *I-B* subregion is a genetic artefact and as such should be removed from the maps of the *H-2* complex. At the same time, the data strongly support the concept that the products of the *Ir* genes are the Ia-bearing molecules.

I-J subregion. Although it is often stated that the *I-J* subregion controls immune suppression⁵¹, we are not aware of any convincing data which support this contention. The experiments with I-J-specific antibodies have only demonstrated that some suppressor T cells express I-J-controlled determinants on their surfaces and that I-J determinants are present on Ts-derived factors. However, this finding could mean that I-J determinants are merely markers of suppressor T cells, just as the so-called

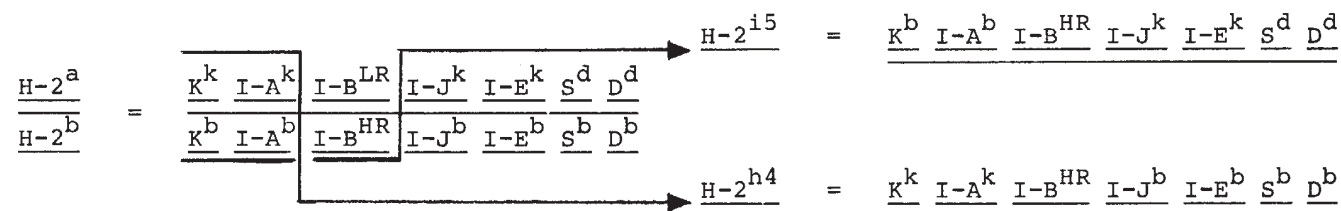


Fig. 1 Definition of the *I-B* subregion by formal genetics. The figure shows the derivation of two *H-2* recombinants, $H-2^{15}$ and $H-2^{h4}$, from an $H-2^a/H-2^b$ heterozygote. To explain the pattern of responsiveness to LDH_B or mouse IgG2a myeloma protein (indicated by the HR and LR symbols which represent 'high' and 'low' responsiveness, respectively), an *I-B* subregion is postulated. The directions of the genetic exchanges leading to the recombinants are indicated by arrows.

Lyt antigens are markers of certain other T-cell subpopulations⁵². It may be, therefore, that the J molecule is unrelated to the A and E molecules; in the absence of hard data regarding the I-J function it is better to remain uncommitted. **I-C subregion.** This portion of the I region was once thought to control serologically detectable molecules⁵³ but this finding could not be substantiated⁵⁴. There are, however, data in the literature which suggest that the I-C subregion controls the generation of suppressor T cells in MLC⁵⁵. Also, the Is genes may require the existence of a separate I-C subregion⁴⁷. However, the evidence for the I-C subregion is so weak that at present it is better to leave the subregion out of the H-2 maps until its existence is fully established.

S region

This chromosomal segment is strikingly different from the rest of the H-2 complex: it does not control any of the traits associated with the K, D or I regions, and complement activity, the only trait that it does control, is not affected by the other regions. This gives the impression that the S region is an alien immigrant into the H-2 complex and has functionally nothing to do with the complex itself. (The argument that the S region is part of the H-2 complex because, like the H-2 proper, it affects immunological functions is absurd because then all loci affecting immunological functions would have to be considered as members of this complex.)

Function of the H-2 loci

We have collected evidence which indicates that each region or subregion of H-2 (with the exception of D) contains only one locus which controls all the traits attributed to this chromosomal segment. The question which then arises is which of the traits reflect the true function of H-2. The answer to this question is not as difficult as it might seem; several of the traits listed in Table 1 are artefacts that cannot possibly have any physiological role. Mice do not normally encounter tissue grafts or lymphocytes from other mice and thus allograft reaction, CML and MLR cannot have any function in an intact individual. On the other hand, immune responsiveness, immune suppression, T-B cell interaction, and the restriction of T-cell specificity are physiologically important phenomena which must be related to the natural H-2 function. Because all these phenomena have a common denominator in that they reflect the recognition by T cells of an antigen in the context of H-2-controlled molecules, the role of H-2 must be to provide this context for antigen recognition. This dual recognition may be the only way for organisms to distinguish non-self from self.

The basis for the Ir-gene phenomenon is the fact that certain combinations of self (H-2 molecules) and non-self (antigen) fail to stimulate T lymphocytes. This failure could have at least two reasons: in some situations the particular antigen/H-2 combination is not recognized by T cells for reasons which we have no space to discuss here; in other situations helper T cells are stimulated but, at the same time, so are suppressor T cells which then prevent the T_h cells from performing their function.

The requirement for simultaneous recognition of antigen and H-2 molecules restricts the specificity of the T lymphocyte—this restriction is probably the real function of the H-2 complex. Molecules known to perform this function are all heterodimers consisting of one highly variable (K, D, L, A_β and E_β) and one relatively constant (β₂-microglobulin, A_α and E_α) polypeptide chain. The variability of K, D, L and A_β chains is generally recognized; that of the E_β chain deserves a special comment. Serological studies have allowed the definition of only three E_β alleles⁵⁶, thus it is often thought that the E_β-encoding locus is less polymorphic than the A_β locus, at which different alleles are known to be present in every independent haplotype tested. However, using a sensitive CML assay, we have recently obtained data which indicate that E_β chains encoded by the different independent haplotypes differ antigenically from one another. This observation suggests that the E_β chains are as variable as the A_β or the K and D chains^{30,31}. The failure to

distinguish the E_β chains by serological methods can probably be attributed to the fact that many independent haplotypes carry the E_α⁰ allele and that the strains bearing this haplotype do not express the E molecule on the cell surface. One should therefore search for haplotype-specific E_β determinants in cells that allow the expression of the E molecule—that is, in appropriate recombinants or H-2 heterozygotes.

The question of how the H-2 molecules actually carry out their function remains largely open. The two principal possibilities are, first, that the H-2 molecules physically interact with the antigen on the antigen-presenting cells and that this interaction then gives the impression of dual specificity of the T cells. Second, that the basis for the dual specificity is the expression by each T cell of two kinds of receptor, one for the antigen and the other for the H-2 molecules⁵⁷. The former hypothesis presupposes a receptor function for the H-2 molecules, a supposition which is not only contradicted by the available data but is also difficult to justify on theoretical grounds. These considerations leave us with the latter hypothesis and hence with the assumption that there are separate T-cell receptors for antigens and H-2; the two receptors, however, need not be distinct.

A simplified view of the H-2 complex

If we accept the proposition that the function of the H-2 complex is to guide T lymphocytes in distinguishing between self and non-self and that the other traits currently attributed to the H-2 molecules are a reflection of this basic function, we arrive at the following model of H-2.

Locus	K A _β A _α E _β E _α D L						
Molecule	45K	28K	32K	28K	32K	45K	45K
Functional class	I	II	II	II	II	I	I

(The order of the loci enclosed in the bracket is unknown. K represents 1,000 MW.) Here the complex is divided into two classes of loci, class II composed of A_α-, A_β-, E_α- and E_β-encoding loci (suitable designations of these loci have not yet been agreed upon). The class I loci differ from those of class II in that the former guide cytolytic and the latter regulatory (helper and suppressor) T lymphocytes, although both classes operate on the same principle.

In this functional model of the H-2 complex, we dispose of regions and subregions and retain only the term 'locus'. The division of the complex into regions and subregions is a historical artefact that confuses the picture, which in reality is probably rather simple. We eliminate from the complex the B locus for which we do not find any evidence, and postpone the inclusion of the J and C loci until convincing data are obtained for their functional relatedness to the complex (J) or for their existence (C). We also exclude from this definition of the H-2 complex the complement-encoding loci which are not functionally related to the class I or class II loci. Although it is useful to list the J and the complement loci as markers on H-2 maps, these loci should not be considered as part of the H-2 complex proper. On the other hand, evidence is accumulating which indicates that the Qa and Tla loci, presently outside the boundaries of the H-2 complex⁵⁸, are in fact part of the complex. Structurally and perhaps also functionally they resemble the class I loci. It is also possible that additional class I loci exist in the vicinity of the K locus and additional class II loci in the A-locus cluster. However, inclusion of these loci in the H-2 map should be postponed until they are clearly established as distinct from all existing loci.

We do not claim that this simplified scheme is the final version of the H-2 complex but we do believe that our interpretation explains the available data much better than the traditional model. We suggest that in H-2 studies the time has come to change the paradigm.

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ARTICLES

Lead isotopic composition of Hercynian granitic K-feldspars constrains continental genesis

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Lead isotope compositions have been determined in many K-feldspars from granitic rocks emplaced during the Variscan (Hercynian) orogeny sensu lato (around 300 Myr) in various parts of Europe and the eastern US. The ratios show a regular geographical pattern with μ contours arched around southern France and parallel to the Atlantic coast of the US. This distribution parallels pre-drift palaeogeographical zones during the Palaeozoic. The lead isotope pattern of Variscan granitic rocks is similar to that of some orogenic volcanic suites but is better explained by crustal melting than by an Hercynian mantle-crust material mixing. The continental crust from these areas must be older than 3,000 Myr. Large scale fluid circulation in the crust during orogenic processes apparently explains the observed geographical patterns of lead isotopes.

WITHIN the general problem of formation and evolution of the continental crust, the nature of the Hercynian (or Variscan) European orogeny has been viewed as resulting from pure ensialic processes^{1,2}, as a former active continental margin³ or as a product of continental collision^{4,5}. An associated parameter is the degree of upper crust-lower crust-mantle material involvement in orogenic magmatism as it gives a direct insight on the nature of source areas affected by significant heat anomalies.

Since the early work of Hurley *et al.*⁶ and Patterson and Tatsumoto⁷, radiogenic isotopes have been shown to be powerful geological tracers for the study of the origin of continental crust. Studies over various geological provinces^{8–11} showed that such an isotopic tracing technique strongly constrains the models proposed for orogenic processes. Here we consider the determination of lead isotopes in K-feldspars of ~300 Myr granitoids from western Europe and the Eastern US.

Because K-feldspars have fairly low U/Pb ratios⁷, their lead isotopic composition is widely assumed to approximate that of the magma from which the crystals precipitated. In addition to allowing comparison with other studies of initial lead the analytical results were expected to provide new information about the geographical patterns shown by lead isotopes in the

Hercynian orogeny and about the behaviour of the U/Pb system in the crust during orogenic processes. (Analytical techniques, experimental results and significant parameters are presented in Table 1.)

Lead isotopic provinces

The isotope ratios of ²⁰⁶Pb/²⁰⁴Pb, ²⁰⁷Pb/²⁰⁴Pb and ²⁰⁸Pb/²⁰⁴Pb vary between 18.04–18.65, 15.55–15.74 and 38.0–39.1 (Table 1, Fig. 1). As pointed out previously^{8,12,13}, the lead isotopic composition of feldspars is virtually independent of rock type in any area except for some rare ²⁰⁶Pb/²⁰⁴Pb variations discussed below.

The three lead isotopic ratios show a clear geographical distribution (Fig. 2), with the most radiogenic samples concentrated in southern France and the least radiogenic in southern Germany, northern France, Cornwall, Morocco and to the west of the Iberian Peninsula. Each of these regions usually gives fairly consistent results at a smaller scale than was observed by Zartman¹³ in the western US.

Anomalously high ²⁰⁶Pb/²⁰⁴Pb ratios and young model ages may be explained by anomalously high U/Pb ratios in the corresponding feldspar samples. The correction for *in situ* U decay from the measured U and Pb concentrations is illusive as U is highly mobile in subsurface conditions^{14,15}, and U/Pb fractionation during sample leaching is an additional source of uncertainty. Within each region, anomalous ²⁰⁶Pb/²⁰⁴Pb ratios

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Table 1 $^{206}\text{Pb}/^{204}\text{Pb}$, $^{207}\text{Pb}/^{204}\text{Pb}$, $^{208}\text{Pb}/^{204}\text{Pb}$ ratios for K-feldspars and plagioclases (Pl) from Hercynian granitoids classified by region and in granulitic–xenolith whole rocks from Massif Central

Sample	²⁰⁶ Pb/ ²⁰⁴ Pb	²⁰⁷ Pb/ ²⁰⁴ Pb	²⁰⁸ Pb/ ²⁰⁴ Pb	Single stage model		Single stage model age (Myr)	Sample	²⁰⁶ Pb/ ²⁰⁴ Pb	²⁰⁷ Pb/ ²⁰⁴ Pb	²⁰⁸ Pb/ ²⁰⁴ Pb	Single stage model		Single stage model age (Myr)
				μ	K						μ	K	
Pyrénées							Morocco						
MI 20	18.400	15.681	38.25	9.645	3.943	282	MI C 42	18.075	15.601	38.15	9.525	4.068	418
MI 13	18.373	15.713	38.59	9.713	4.120	339	MA 42	18.081	15.610	38.19	9.543	4.085	425
	18.386	15.705	38.59				French Massif Central						
	18.365	15.695	38.55				Lz 3	18.393	15.658	38.37	9.600	3.996	260
MI 12	18.380	15.694	38.56	9.673	4.094	312	Lz 12	18.339	15.644	38.30	9.579	3.989	280
MI 22	18.580	15.708	38.57	9.679	3.987	187	Lz 8	18.398	15.660	38.47	9.604	4.038	259
MI 34	18.447	15.704	38.58	9.687	4.069	278	MLV	18.337	15.683	38.48	9.657	4.080	329
MI 57	18.420	15.692	38.54	9.665	4.062	281	Marg	18.385	15.695	38.55	9.675	4.087	310
MI 32	18.453	15.717	38.66	9.711	4.101	288	Peyr	18.245	15.638	38.37	9.578	4.077	341
	18.469	15.719	38.60				1189	18.193	15.653	38.33	9.615	4.091	396
	MI 66 Pl	18.472	15.695	38.51	9.665	4.019	248	1356	18.212	15.640	38.30	9.592	4.065
	18.459	15.702	38.54				1355	18.258	15.688	38.60	9.676	4.184	391
MI 64 Pl	18.436	15.710	38.60	9.699	4.083	292	1354	18.246	15.654	38.36	9.610	4.072	359
Qt 04 Pl	18.398	15.673	38.46	9.630	4.034	274	Br 1	18.445	15.724	38.59	9.725	4.077	302
Qt 04	18.428	15.683	38.48	9.646	4.028	263	SIDO	18.383	15.684	38.45	9.653	4.043	298
Qt 08	18.455	15.671	38.54	9.671	4.100	263	Iberian Peninsula						
Qt 18	18.428	15.718	38.64	9.715	4.050	307	RUG	18.253	15.623	38.26	9.547	4.016	318
Qt 12	18.401	15.682	38.47	9.647	4.043	283	FIN	18.181	15.616	38.32	9.542	4.086	361
Q 71	18.448	15.693	38.48	9.664	4.018	263	RUF	18.147	15.609	38.23	9.532	4.062	377
Q 15	18.650	15.751	38.76	9.756	4.044	190	PIN	18.565	15.643	38.27	9.553	3.854	117
SL 4	18.455	15.723	38.61	9.722	4.083	294	GC 37	18.230	15.623	38.23	9.552	4.019	334
CAD 1	18.614	15.693	38.66	9.646	4.008	144	M 181	18.265	15.619	38.14	9.538	3.957	304
AX 5	18.660	15.745	39.14	9.743	4.200	175	M 317	17.600	15.483	37.45	9.345	3.991	620
X 1	18.530	15.707	39.00	9.683	4.206	221	M 379	18.174	15.587	38.13	9.485	3.996	331
SVZ	18.342	15.654	38.43	9.598	4.051	291	M 66	18.293	15.630	38.30	9.556	4.013	297
Brittany—Cornwall							M 254	18.254	15.641	38.24	9.583	4.014	338
Pc 5630	18.355	15.611	38.27	9.512	3.961	230	M 247	18.268	15.630	38.22	9.559	3.994	315
Pc 5615	18.256	15.594	38.15	9.489	3.960	280	Corsica						
Pc 5618	18.218	15.603	38.15	9.511	3.984	319		18.300	15.628	38.32	9.552	4.019	290
Pc 561	18.221	15.600	38.11	9.505	3.963	313	Appalachians						
Brt 17	18.225	15.619	38.28	9.543	4.042	330	Mil 1	18.370	15.640	38.28	9.567	3.962	254
Brt 5	18.212	15.621	38.30	9.548	4.058	344	Mil 2	18.372	15.633	38.28	9.553	3.959	244
Fla	18.301	15.591	38.13	9.478	3.925	244	1372	18.344	15.631	38.18	9.552	3.930	262
WR	18.399	15.624	38.33	9.532	3.966	214	GA 4B	18.345	15.590	38.04	9.471	3.860	211
RC 4	19.786	15.690	38.30				Ly 3	18.486	15.634	38.39	9.543	3.945	163
Vosges—Black Forest							1285	18.377	15.601	38.11	9.486	3.873	201
STA	18.093	15.565	38.03	9.451	3.994	363	1302	18.385	15.607	38.14	9.500	3.883	203
STB	18.141	15.558	38.00	9.431	3.950	320	1141	18.481	15.602	38.12	9.481	3.820	127
Hck	18.125	15.573	38.05	9.463	3.984	349	Granulitic xenoliths (whole rocks)						
CDF	18.039	15.553	38.01	9.434	4.010	387	2520	18.530	15.645	38.73			
SAUL	18.155	15.612	38.21	9.537	4.051	374	803	18.190	15.663	38.49			
FN 3	18.095	15.585	38.06	9.491	4.010	385	509	18.737	15.687	38.75			
FN 12	18.200	15.615	38.25	9.538	4.041	346	802	18.535	15.693	39.10			
FN 1	18.025	15.583	38.08	9.496	4.060	433	199	18.637	15.676	38.58			
FN 13	18.140	15.601	38.12	9.517	4.015	372	168	18.266	15.670	38.50			
							BAL 1	18.697	15.727	39.35			

The single stage model, μ , K and model age are calculated from the modern lead values given by Stacey and Kramers¹⁶ and initial lead values of Tatsumoto *et al.*¹⁷. The analytical technique for lead separation is described in ref. 58 and the leaching procedure in ref. 56. Analytical uncertainties are reproducible from one measurement or another and are in the 1% range.

Provenance of granite samples:

Pyrénées—MI, Maladetta; Qt, Q, Querigut; SL, Saint Laurent; CAD, Cady; AX, X, Ax les Thermes; SVZ, Salvezines.

Brittany—Pc, Ploumanach; Brt, south Brittany; Fla, Flamanville.

Cornwall—Wr, saint Austell; RC, Tregonning.

Vosges, Black Forest—STA, STB, Remirmont; HCK, Honeck; CDF, Champ du Feu; SAUL, Saulieu; FN, Black Forest.

Morocco—M 11, MA, Zeida.

Massif Central—Lz, Mt Lozère; MLV, Millevache; Marg, Margeride; Pey, Peyrol; 1189, St Mathieu; 1356, Brême; 1355, 1354, St Sylvestre; Br, Brousse; SIDO, Sidobre.

Iberian Peninsula—RUG, FIN, RUF, PIN, Galicia; GC, Guadarrama; M, Sierra Morena.

Corsica—Vizavonna.

Appalachians—Mil 1 and Mil 2, Milford (NH); 1372, York (SC); GA 4B, Sparta (GA); Ly 3, Lyman (ME); 1285, Lilesville (NC); 1302, Pageland (SC); 1141, Castalia (NC).

All granulitic xenoliths come from Bournac (Massif Central).

roughly correlate with high U/Pb ratios (Table 2). For most of the 'normal' samples the measured $^{238}\text{U}/^{204}\text{Pb}$ values are <1 , and therefore, for a given region, the least radiogenic samples should be close to the initial lead isotope composition of the parent magmas.

Three samples exhibit anomalously high $^{207}\text{Pb}/^{204}\text{Pb}$ values when compared with their expected regional value. All of these samples, Qt-15 (Querigut, Pyrénées), AX5 (Ax les Thermes, Pyrénées) and 1355 (Saint Sylvestre, Western Massif Central), present strong evidence of deuterium effects. Although the cause of the observed shift is unknown, it is probably related to country-rock interaction or to inherited U-rich mineral leaching. When applied to these samples, the single stage model using the modern lead isotopic composition of Stacey and Kramers¹⁶

gives an age in the range 360–260 Myr which is in reasonable agreement with their actual age. Therefore we used this model together with the primordial lead values of Tatsumoto *et al.*¹⁷ to estimate the μ values of the reservoir for each sample.

The apparent μ values vary from 9.45 (Vosges) to 9.70 (Pyrénées) and straddle the μ value (9.52) calculated for the so-called 'normal growth curve', whose parameters are used here.

Apparent $K(^{232}\text{Th}/^{238}\text{U})$ values range from 3.94 to 4.10 (discarding the samples with anomalous $^{206}\text{Pb}/^{204}\text{Pb}$ ratios) but show no obvious geographical pattern.

The systematic variation of lead isotope composition across the Hercynian orogeny is shown in Figs 2 and 3. A plot of the apparent μ values on a map (Fig. 3) with the Iberian Peninsula

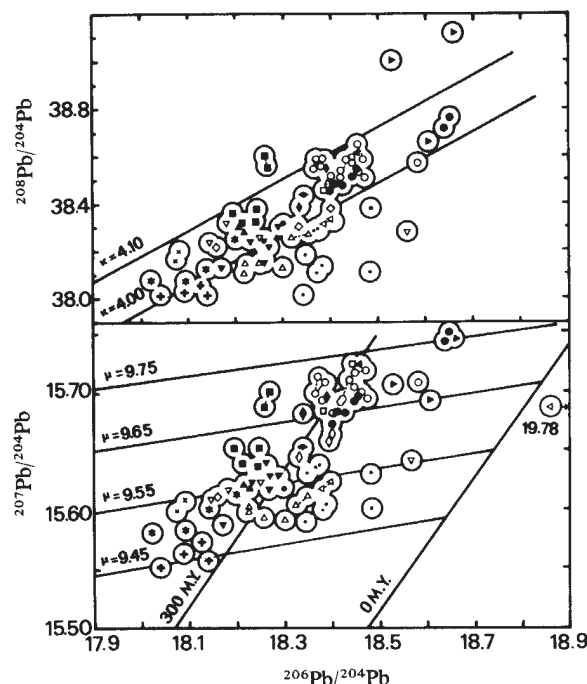


Fig. 1 Analytical data for Western Europe and eastern North America plutonic feldspars. Single stage evolution curves and isochrons are calculated from data in refs 16, 17. Different symbols indicate different geographical areas.

and Corsica in their predrift position^{18,19}, illustrates two fundamental points: a pattern of concentric zones centred on southern France with values decreasing outwards and apparently monotonous and continuous variations of the μ values. Palaeogeographical boundaries inferred for the Devonian¹⁹ are also shown on Fig. 3 and their agreement with the zones is remarkable. Moreover, the position of the palaeogeographical domains did not change very significantly during most of the Palaeozoic.

The least radiogenic lead isotope compositions are located on the 'outer' (northern or western) edges of the orogeny (zone 3 of Fig. 3) where prominent Palaeozoic volcanism (spilites, dolerites and dominant keratophyre/rhyolites) is present^{20,21}. In contrast, the magmatic and tectonometamorphic 'crest' running from Galicia through southern Brittany and Northern Massif Central to Central Europe²² does not show in the lead isotope distribution. It has been proposed^{5,23,24} that the prominent Hercynian slip fault running along this line could be the remnant of an intercontinental suture through which oceanic lithosphere (whose width has been estimated²² to ~2,000 km) has been consumed during Lower and Middle Palaeozoic. Indeed, the only three known occurrences of Hercynian blue-amphibole have been reported along this line as well as rocks in the eclogite facies and true Hercynian peridotites. The present data do not support a discontinuity in the lead isotope composition across this boundary, though allowance must be made for the scale of our sampling and the effect of analytical uncertainties which may blur the observed variations. The very similar lead isotope compositions in granitic feldspars from Brittany and Galicia add another piece of evidence for the post-Hercynian opening of the Bay of Biscay²⁵.

We have also analysed a few samples from the eastern US. The K-feldspars from Hercynian rocks there have distinctive lead isotope compositions: $^{206}\text{Pb}/^{204}\text{Pb}$, 18.34–18.48; $^{207}\text{Pb}/^{204}\text{Pb}$, 15.59–15.64; $^{208}\text{Pb}/^{204}\text{Pb}$, 38.05–38.39 (Fig. 1). In the isotope diagram these compositions define a domain adjacent to, but significantly different from the domain for the European Hercynian granites. Both the $^{207}\text{Pb}/^{204}\text{Pb}$ ratios and the apparent μ values calculated for the source of granitic rocks decrease to the south-east when moving across the major geological boundaries, which suggests a pattern broadly similar

to the one observed in Europe. Current strontium and oxygen isotope analyses of Hercynian granites from the eastern US^{26–30} show that despite much lower initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratios (0.703–0.706), these rocks have kept the $\delta^{18}\text{O}$ record of a previous alteration–sedimentation cycle. A crustal origin previously proposed for these rocks is confirmed by the continental signature of the lead isotopes. However, any attempt to rebuild a predrift model for the Hercynian orogeny, isotope data requires that the eastern US and Europe are distinctive segments of the Palaeozoic continental crust.

Genesis of Variscan crust according to Pb isotope data

On a $^{206}\text{Pb}/^{204}\text{Pb}$ – $^{207}\text{Pb}/^{204}\text{Pb}$ diagram, the Variscan granitoids fall into the future (J) lead domain relative to single stage model calculated with the initial lead data of Tatsumoto *et al.*¹⁷ and the U decay constants of Jaffey *et al.*³¹. Figure 4 shows the Hercynian granitoid domain (shifted 300 Myr forwards) and that for Mid-Ocean-Ridge basalts. It is impossible to make these two domains coincide. The model generating granitoids through partial melting of a subducting plate or through melting of mantle-type igneous rocks accumulated in an interarc basin, does not apply to Variscan granitoids from Europe and the eastern US. Even considering a period of the order of 10^9 yr, between the time of formation of the basalt series and that of their melting, it is impossible to generate the Hercynian granitoid domain, the apparent μ of the latter being much too high. However, the Hercynian granitoid domain (with isotopic compositions corrected for decay that would have occurred in source rocks during 300 Myr) compares well with high $^{207}\text{Pb}/^{204}\text{Pb}$ samples from the Caribbean Islands (ref. 32 and B. Dupre, unpublished results), while being markedly different to results from other areas, such as the Cascades³³, the Mariana Islands³⁴ or the Andes³⁵.

To explain the lead isotopic composition of Variscan granitoids, we may consider two extreme models on the basis of geological and geochemical observations.

(1) The crust–mantle mixing model: The granitoids are derived from a mixture of mantle and recycled preexisting continental crust^{36,37}.

The present lead isotopic data for Hercynian granitoids are compatible with this model. Values for lead of oceanic origin are variable^{38,39} and the continental crust is heterogeneous. We may thus expect that a mixture of these two heterogeneous components could yield the observed pattern. However, as far as lead isotopes are concerned, the mantle can be of the oceanic ridge type but is unlike the source of ocean-island basalts or of continental flood-basalts. Note that this model is not based on a mixture of one crustal component and one mantle-derived component but on a mixture on an unspecified number of components of each type.

Table 2 Concentrations of Pb and U (ID) as determined by isotope dilution, U and Th by neutron activation

Sample	Pb (p.p.m.)	U (p.p.m.)	Th (p.p.m.)	$^{238}\text{U}/^{204}\text{Pb}$	$^{232}\text{Th}/^{238}\text{U}$
FIN	69.5	0.41 0.427 (ID)	1.52	0.5	3.70
PIN	33.8	5.57 5.52 (ID)	2.93	14	0.52
RUG	52.5	1.55 1.762 (ID)	6.11	2.6	3.94
CdF	24.6	0.20 0.196 (ID)	1.65	0.6	8.25
Qt 15	42.7	0.33	0.85	0.7	2.57
Qt 71	46.15	0.41	1.25	0.7	3.08
Ly 3	52.8	0.34	0.84	0.5	2.47
GA 4B	31.6	0.23	0.77	0.6	3.34
WR	60.24	0.23 0.203 (ID)	0.57	0.3	2.47
RC4	7.7	0.84 0.97 (ID)	0.137	11	0.16

J. L. Joron analyst, Laboratoire Pierre Süe.

(2) The crust-derived granitoid model: Here, granitoids are derived from a U- and Pb-zoned continental crust. Direct geochemical evidence⁴⁰, heat flow studies^{41,42} as well as earlier isotopic studies⁴³⁻⁴⁵ have suggested that the lower crust is depleted in U relative to the upper crust and should be characterized by significantly lower μ values. This model implies that granites with low μ values would derive from the lower crust and those with higher μ values from the intermediate crust. As a corollary to this model, ancient source rocks are indicated by the average slope of the $^{207}\text{Pb}/^{204}\text{Pb}$, $^{206}\text{Pb}/^{204}\text{Pb}$ pattern (Fig. 3) (about 3,800 Myr for a secondary isochron). Even if such a figure does not accurately reflect the local primitive differentiation age of the crust—a point which could be really difficult to confirm—the initiation of these μ differences must be ancient (>3,000 Myr) to produce the observed $^{207}\text{Pb}/^{204}\text{Pb}$ variations.

Before discussing these models, we must point out that they are neither mutually exclusive nor at variance with more complex models which involve continuous or episodic mixing and reworking cycles^{46,47}. The contamination discussed in

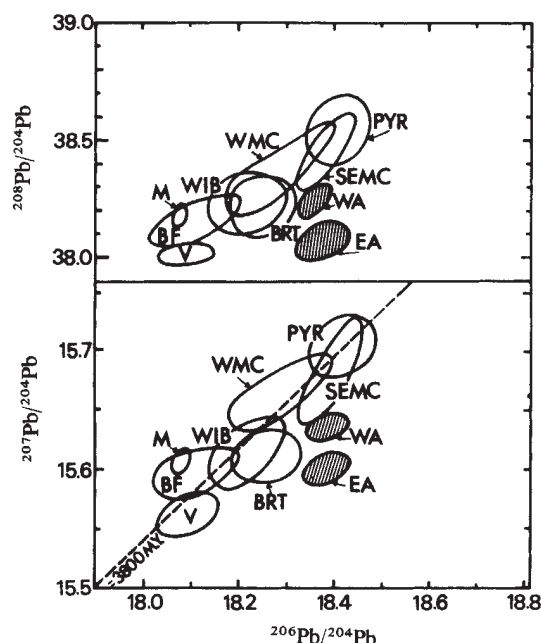


Fig. 2 Distribution of data according to their geographical origin. A few anomalous radiogenic samples have been discarded. PYR, Pyrénées; WMC, western Massif Central; SEMC, southeastern Massif Central; WIB, west of the Iberian Peninsula; BRT, Brittany and Cornwall; M, Morocco; BF, Black Forest; V, Vosges. Hatched fields: WA, Western Appalachians; EA, Eastern Appalachians.

model (1) can take place at different levels of the crust and thus the contaminating component may have highly variable μ values including very low ones. The problem is thus to determine when and where the crust-mantle mixing occurred. The following arguments seem to favour a crustal origin rather than a crust-mantle mixing in Hercynian time.

(1) The measured isotopic compositions for neodymium³⁷, strontium⁴⁸⁻⁵² and oxygen⁵³ in similar granitoids indicate that the granite source is the continental crust and not the mantle. (2) We have measured the lead isotopic composition for seven granulite-facies xenoliths from Bournac, France, for which preliminary data on zircons suggest an age of ~300 Myr (Table 1). These specimens are a representative sample of the catagone for the French Massif Central^{54,55}. No accurate correction for uranium decay during the past 300 Myr can be applied to the data due to the lack of U and Pb concentrations for these particular samples, so their field is shown in Fig. 4 without correction. Nevertheless, the assumption of μ values in a range reasonable for this rock type (0–5), can obviously make the domains of granulitic xenolith and Hercynian granitic rocks from the same area coincide, thus permitting a genetic link between them. Although this coincidence is only shown for the

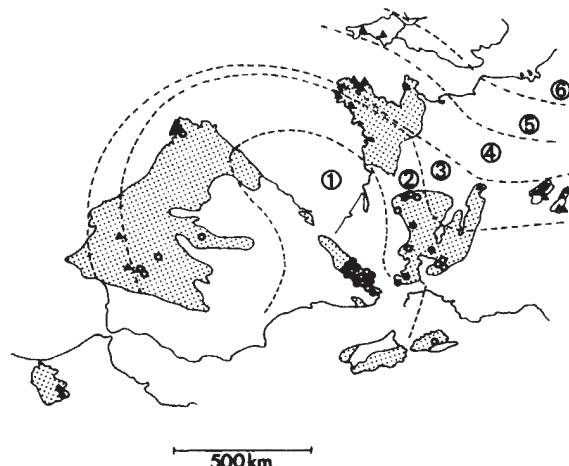


Fig. 3 Geographical plot of single stage μ values for plutonic feldspar in Western Europe on a predrift reconstruction proposed by Le Pichon¹⁸. Approximate palaeogeographical boundaries (dashed line) and palaeogeographic zones numbered 1 to 6 (6 is the stable Europe) are simplified from Matte¹⁹. Model μ values: ●, >9.65; ○, 9.55–9.65; ▲, 9.45–9.55; ◆, <9.45. □, Prehercynian outcrops.

Massif Central we suggest that it can be extended to areas of distinct $^{207}\text{Pb}/^{204}\text{Pb}$ values.

(3) The very existence of isotopic provinces is better understood in such a model as these provinces are likely to reflect variations in orogenic segment characteristics (thickness, chemical and petrological compositions, age). In this way, the isotopic characteristics are expected to parallel the geological provinces defined from structural observations and metamorphic petrology¹⁹.

(4) In the crust-mantle mixing model, we could anticipate that on a regional scale, Vosges or Cornwall granitoids should present a much more pronounced mantellic signature than those from the southern France. Petrographic and geochemical evidence for such a distinction is virtually absent.

Consequences for the orogenic processes

The present results suggest that Variscan granitoids were generated principally (if not totally) by remobilizing the previously differentiated continental crust. In western Europe and in the eastern US, at least, no new segment of continental crust has been formed during the Hercynian event. Such a result agrees with recent Sm–Nd and Rb–Sr data³⁷ according to which very little continental crust has been formed in recent time and the young crust results from a mere recycling of preexisting

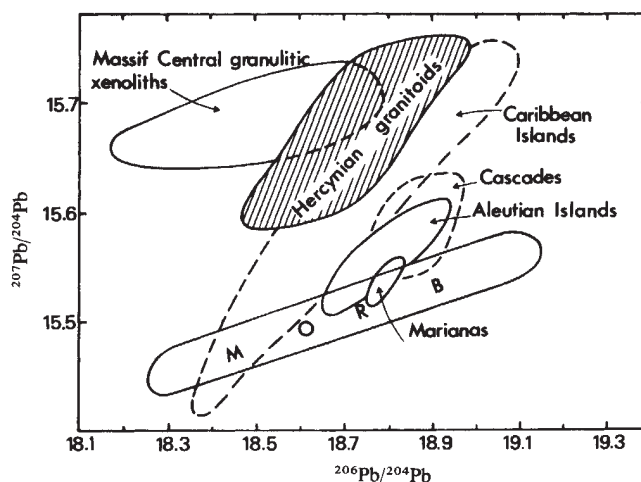


Fig. 4 $^{207}\text{Pb}/^{204}\text{Pb}$ versus $^{206}\text{Pb}/^{204}\text{Pb}$ fields of some present day volcanic suites: MORB (Mid-Ocean-Ridge Basalt)⁵⁹, Marianas³⁴, Cascades³³, Caribbean (ref 32 and B. Dupre, unpublished results), Aleutian⁶⁰, compared with the field of Hercynian plutonic feldspar data shifted 300 Myr forwards in time and to that of granulitic xenoliths from Massif Central.

continental material, whereas the interpretation of lead and strontium isotope data may differ for the Precambrian.

A second point concerns the behaviour of lead isotopes during the genesis of granitoid rocks. Although strontium and oxygen isotopic compositions may vary even within a single pluton⁵⁶, lead isotopes are remarkably uniform on a regional scale. As observed for the Maladeta intrusion⁵⁶, and at Ploumanac'h (Brittany), the lead isotopic ratios are virtually indistinguishable among different rock types from a single composite pluton (gabbro, granodiorite, granite). In our opinion, this requires their being levelled at the local average for the crustal lead by fairly efficient transport and/or reequilibration mechanisms. This observation implies that a large scale circulation of fluid does exist during such processes, remobilizing and homogenizing lead isotopes, much more than strontium or oxygen.

Such an important circulation of fluids during melting of the lower crust may be responsible for the zoning of radioactive elements⁵⁷. It may also induce remobilization and enrichment in

incompatible elements such as Pb, Zn, Mo, Ta, Sn, U of the continental crust. The orogenic remobilization may be the origin of ore deposits associated with granitoids thereby yielding the so-called metallogenic provinces. When distinctive regional chemical characteristics exist, they are perpetuated through geological time through recycling and fluid remobilization processes. In the present view, lead isotopic provinces and metallogenic provinces will coincide as both result from a pervasive fluid circulation within the Earth's crust.

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Only one of two closely related yeast suppressor tRNA genes contains an intervening sequence

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The yeast genes that code for the serine-inserting SUP-RL1 amber and SUQ5 ochre suppressors have been cloned and sequenced. These two unlinked genes differ by only three base pairs in their coding regions yet they encode tRNAs of different translational specificities, and while the SUP-RL1 gene has a 19-base pair intervening sequence, the SUQ5 gene has none.

SINCE the selection of yeast nonsense suppressors was first described by Hawthorne and Mortimer¹, the study of these genes has contributed to several significant advances in eukaryotic molecular genetics. Yeast suppressor strains, for example, were used to prove that eukaryotes and prokaryotes use the same nonsense codons^{2,3}; they were the source of transfer RNA in the first demonstrations of *in vitro* suppression in eukaryotic

translation systems^{4,5}; they were the first eukaryotic mutants to be sequenced both at the levels of RNA⁶ and DNA⁷, and they played a part in the discovery of intervening sequences and RNA splicing^{7–10}.

Most work on yeast nonsense suppressors has involved the tyrosine and serine-inserting suppressor genes. The tyrosine-inserting suppressors derive from a set of eight unlinked tyrosine

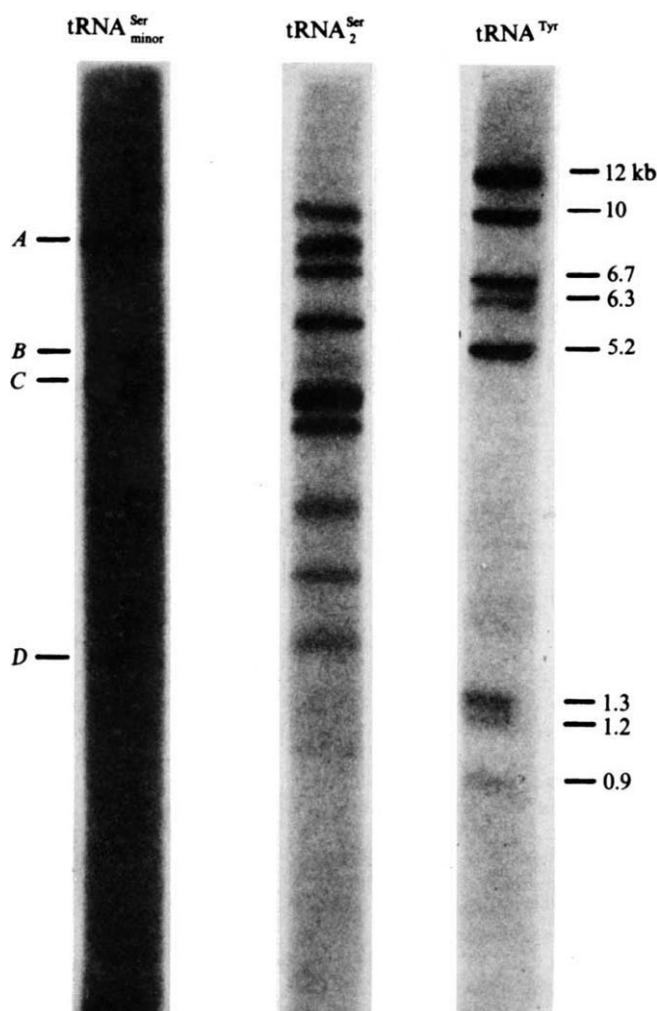


Fig. 1 Hybridization of three different ^{125}I -labelled yeast tRNA probes to *Eco*RI-cleaved, gel-fractionated yeast DNA. Yeast DNA from the strain Y4A was cleaved with *Eco*RI and fractionated on an agarose gel before being transferred to nitrocellulose⁴⁹. Preparation of yeast DNA, electrophoresis, labelling of the tRNAs and hybridization were all as previously described^{11,13}. The tRNA^{Ser_{minor}} mixture was fractionated on and eluted from a two-dimensional acrylamide gel^{15,23}. The purification of tRNA^{Tyr} and tRNA^{Ser₂} has also been described elsewhere^{11,34}. The sizes of the four tRNA^{Ser_{minor}}-hybridizing fragments are ~8.3, 4.7, 4.3 and 1.5 kilobases (kb).

tRNA genes, all of which code for the same mature tRNA. Reports are available on the cloning and sequencing of both wild-type and mutant tRNA^{Tyr} genes^{7,11,12} as well as on the cross-correlation of the physical and genetic descriptions of the entire eight gene family¹³.

The yeast serine-inserting suppressors derive from a family of tRNA genes that is both less studied and more complex than the tyrosine case. There are six serine codons (UCC, UCU, UCA, UCG, AGU and AGC), which are translated by at least four different classes of serine tRNAs^{14–17}. Genetic analysis has revealed three different loci that can mutate to serine-inserting nonsense suppressors: the recessive lethal *amber* suppressor *SUP-RL1* (chromosome III)^{8,18} and the *ochre* suppressors *SUQ5* (chromosome XVI)^{19–22} and *SUP17* (chromosome IX)¹⁹.

SUP-RL1 was shown by Piper¹⁵ to cause the alteration CGA→CUA in the anticodon of a unique component of a heterogeneous set of minor serine tRNAs, which we designate tRNA^{Ser_{minor}}. It was recently demonstrated that the *sup-RL1*⁺ gene product is the only component of tRNA^{Ser_{minor}} whose anticodon is complementary to UCG, and a precursor to this tRNA containing a 19-base pair intervening sequence was also characterized¹⁶.

We have performed a molecular analysis of the serine-inserting suppressor genes starting with a hybridization probe made

by labelling the tRNA^{Ser_{minor}} mixture. Here we demonstrate that the tRNA^{Ser_{minor}} species are transcribed from a family of four genes, all of which have been cloned from a wild-type yeast strain, and two of which have been identified with the genetic loci *SUP-RL1* and *SUQ5*. DNA sequences have been determined for wild-type and suppressor alleles of both genes. One striking feature of the results is that although the *SUP-RL1* and *SUQ5* genes differ by only three base pairs within their coding regions, the *SUP-RL1* gene has an intervening sequence whereas the *SUQ5* gene does not.

The tRNA^{Ser_{minor}}s are encoded by a four-gene family

To characterize and clone the genes for the various tRNA^{Ser_{minor}} species, we used ^{125}I -labelled tRNA^{Ser_{minor}} as an RNA-DNA hybridization probe. The tRNA^{Ser_{minor}} mixture was purified by two-dimensional gel electrophoresis²³ and then iodinated by a procedure that specifically labels the modified nucleoside isopentenyl adenosine¹¹. The presence of this nucleoside in all the components of tRNA^{Ser_{minor}} offers a convenient means of labelling these RNAs efficiently and relatively specifically.

The number of genes in the tRNA^{Ser_{minor}} family was estimated by examining the hybridization of ^{125}I -labelled tRNA^{Ser_{minor}} to *Eco*RI-cleaved, size-fractionated yeast DNA. As shown in Fig. 1, there are four different yeast *Eco*RI fragments that hybridize to tRNA^{Ser_{minor}}. Comparison of the tRNA^{Ser_{minor}} hybridization pattern with that produced by ^{125}I -labelled tRNA^{Ser₂} indicates that at least three and probably all four of the tRNA^{Ser_{minor}}-hybridizing *Eco*RI fragments are distinct from those that hybridize to tRNA^{Ser₂}.

All four of the tRNA^{Ser_{minor}}-hybridizing *Eco*RI fragments were cloned in the λ insertion vector 607 (ref. 24). Plaques from a pool of λ -yeast recombinants, prepared by ligating together a mixture of *Eco*RI-cleaved λ and total yeast DNA, were screened using ^{125}I -labelled tRNA^{Ser_{minor}} as the hybridization probe. Recombinant λ were isolated containing yeast *Eco*RI fragments that were identical in size to each of the four fragments A–D. Both the recombinant λ plaques and the cloned *Eco*RI fragments themselves showed strong hybridization to ^{125}I -labelled tRNA^{Ser_{minor}}.

Chromosomal location of two of the genes

Various RNA-DNA and DNA-DNA hybridization experiments were carried out to identify one or more of the tRNA^{Ser_{minor}}-hybridizing *Eco*RI fragments with known suppressor loci. We examined the hybridization of ^{125}I -labelled tRNA^{Ser_{minor}} and some of the ^{32}P -labelled cloned *Eco*RI fragments to DNA from yeast strains that were aneuploid for chromosomes to which serine-inserting suppressor loci have been mapped. Strains that are disomic for a single chromosome ($n+1$) are particularly useful in this type of analysis. In the simplest case, one member of a set of bands produced by a particular probe may have enhanced intensity when DNA from a specific disomic strain is used. This behaviour implies that this band arises from hybridization to a restriction fragment that is derived from the disomic chromosome. Alternatively, a particular sequence that hybridizes to the probe may exist on restriction fragments of different sizes on the two homologous chromosomes present in the disome; in this case an extra band appears in hybridizations to disomic DNA. Both of these situations have been observed before^{13,25} and were encountered again here.

The first disomic strain that was examined contained an extra copy of chromosome III, the chromosome to which the *SUP-RL1* locus maps. Figure 2a shows that, compared with a haploid control, an enhanced C band is observed when ^{125}I -labelled tRNA^{Ser_{minor}} hybridizes with *Eco*RI-cleaved DNA from the chromosome III disome. This result establishes the tRNA^{Ser_{minor}} gene on the C fragment as a candidate for the *SUP-RL1* gene. The same DNA filter that was used for the ^{125}I -labelled tRNA^{Ser_{minor}} hybridization was subsequently used in hybridization with ^{32}P -labelled DNA from the cloned C fragment. Once

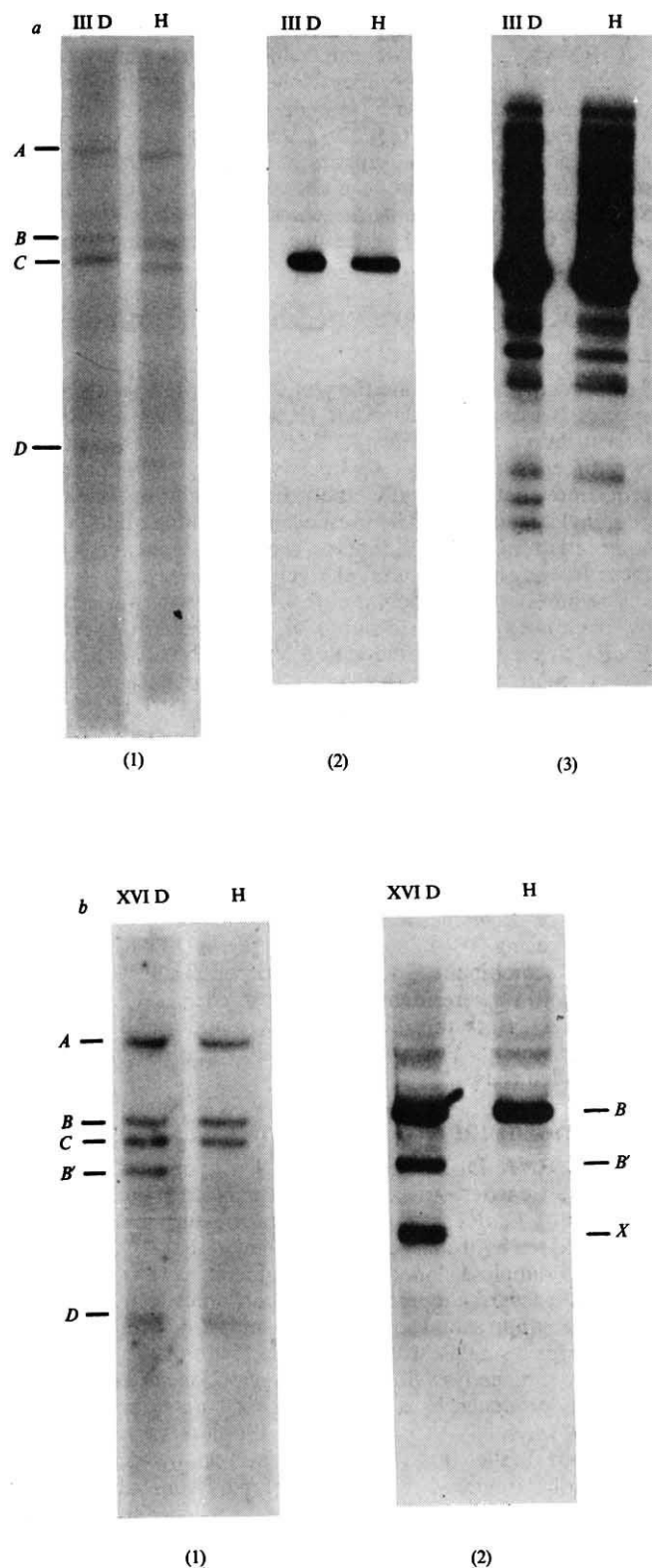


Fig. 2 Hybridization of ^{125}I -labelled $\text{tRNA}^{\text{Ser}}_{\text{minor}}$ and ^{32}P -labelled fragment-specific probes to DNA from aneuploid and haploid yeast strains. *a*, Comparison of the hybridization of ^{125}I -labelled $\text{tRNA}^{\text{Ser}}_{\text{minor}}$ (1) and ^{32}P -labelled cloned *C* fragment (2 and 3) with DNA from a chromosome 3 disome and a haploid yeast strain. The photograph in (3) is a longer exposure of the same autoradiogram as in (2). The haploid strain (96C) was derived from the disome (96CH) by mitotic chromosome loss. *b*, Comparison of the hybridization of ^{125}I -labelled $\text{tRNA}^{\text{Ser}}_{\text{minor}}$ (1) and ^{32}P -labelled cloned *B* fragment (2) with DNA from a chromosome XVI disome and a haploid yeast strain. The haploid strain (DH4093-5DH) was derived from the disome (DH4093-5D) by mitotic chromosome loss. (Both strains were provided by D. C. Hawthorne.) Experimental details were as described in Fig. 1 legend except that the hybridization conditions for the nick-translated, agarose gel-purified restriction fragments were as defined elsewhere¹³.

again, an enhanced *C* band was observed with the chromosome III disome DNA. On longer exposure of the autoradiogram, it was also observed that the ^{32}P -labelled *C* fragment hybridizes quite strongly with a large number of other *Eco*RI fragments, presumably because of the presence on this fragment of one or more dispersed repetitive sequences.

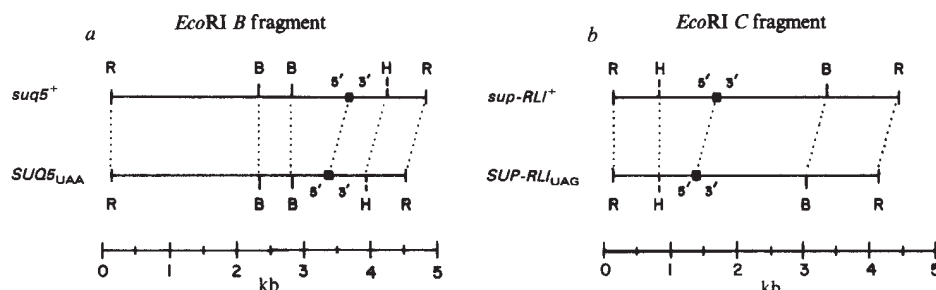
Similar experiments using DNA from a chromosome XVI disome allowed a tentative identification of the $\text{tRNA}^{\text{Ser}}_{\text{minor}}$ gene on the *B* fragment with the serine-inserting suppressor locus *SUQ5*. In this case, however, instead of enhancement of the intensity of a pre-existing band, a new band appeared in the hybridization pattern of the disomic strain (Fig. 2*b*). This behaviour would be consistent with the possibility that one of the three unmapped *Eco*RI fragments (*A*, *B* or *D*) derives from chromosome XVI and that it exists in two variant forms on the disome's two copies of this chromosome. Because there was no *a priori* basis on which to associate the new band with a particular one of these three fragments, we performed hybridization experiments with the ^{32}P -labelled cloned *A*, *B* and *C* *Eco*RI fragments to identify the new band.

The first case tested, the *B* fragment, was found to have extensive homology with the disome's new $\text{tRNA}^{\text{Ser}}_{\text{minor}}$ -hybridizing *Eco*RI fragment. When DNA from the disome was used, the ^{32}P -labelled *B* fragment hybridized strongly to three *Eco*RI fragments, labelled *B*, *B'* and *X* in Fig. 2*b*. *B'* is identical in size to the 'extra' ^{125}I -labelled $\text{tRNA}^{\text{Ser}}_{\text{minor}}$ fragment observed with disome DNA, while *X* is a fragment that does not hybridize to the tRNA. In the haploid, ^{32}P -labelled *B* hybridizes strongly only to the normal *B* fragment. These results indicate that in the disome one of the chromosome XVI homologues contains *B* fragment sequences on the two variant fragments *B'* and *X*, with the $\text{tRNA}^{\text{Ser}}_{\text{minor}}$ coding region lying on *B'*. The results in Fig. 2*b* also show that the cloned *B* fragment, like *C*, contains one or more dispersed repetitive elements.

Although the experiments on the chromosomal origins of *B* and *C* suggested the tentative assignments *B* = *SUQ5* and *C* = *SUP-RL1*, more direct evidence was required before unequivocal assignments could be made. Our next goal, therefore, was to determine the DNA sequences of the $\text{tRNA}^{\text{Ser}}_{\text{minor}}$ genes on *B* and *C*, as cloned from both wild-type and suppressor yeast strains. The details of the re-cloning of the *B* and *C* fragments from suppressor strains are described in Fig. 3 legend. Although the re-cloning experiments were basically straightforward, two minor points required special attention: first, the presence of dispersed repetitive DNA on both the *B* and *C* fragments complicated the choice of *B*- and *C*-specific DNA-DNA hybridization probes, and second, the fact that *SUP-RL1* can only be maintained in the heterozygous state required that potential *SUP-RL1*_{UAG} clones be isolated from a λ -yeast recombinant phage pool that contained equal numbers of clones of the *SUP-RL1*_{UAG} and *sup-RL1*⁺ alleles. The first problem was solved by identifying subfragments of *B* and *C* that contain little or no dispersed repetitive DNA, and then using plasmids containing these predominantly single-copy subfragments as specific hybridization probes. Our approach to the second problem was to screen λ clones of the *C* fragment from a *SUP-RL1*_{UAG}/*sup-RL1*⁺ heterozygote for a clone that was lacking the *Taq*I site that is present in the tRNA coding region of the wild-type gene. On the basis of the tRNA sequence, this site is predicted to be eliminated by the mutation of *sup-RL1*⁺ to *SUP-RL1*_{UAG}.

One unexpected finding during the re-cloning experiments was that the *B* and *C* fragments were both a few hundred base pairs smaller in the suppressor strains than in our original wild-type strain. As shown in Fig. 3, the missing DNA in the suppressor strains in both cases maps to a region near the 5' end of the tRNA coding region. It should be emphasized that the suppressor strains were laboratory stocks that were not closely related to the original 'wild-type' strain. As a high degree of strain-to-strain variation in restriction fragment sizes has been previously documented for tRNA^{Tyr} -hybridizing restriction fragments¹³, the mere presence of this variation among

Fig. 3 Restriction maps of the tRNA^{Ser}-hybridizing *EcoRI* fragments *B* and *C*. *B*, *Bam*HI; *H*, *Hind*III; *R*, *Eco*RI; ■, tRNA coding region. *a*, The map labelled *suq5*⁺ refers to the original clone (APM15) of the *EcoRI B* fragment from the wild-type yeast strain Y4A. The 1.4-kilobase (kb) *Bam*HI/*Hind*III subfragment was transferred to the plasmid vector pBR322 for fine structure restriction mapping and DNA sequencing. This plasmid (pPM15) was also nick-translated and used to screen a new λ607 pool of yeast *EcoRI* fragments, derived from the *SUQ5-ochre* strain 4093-4A (obtained from D. C. Hawthorne). In this way the new *EcoRI B* fragment, labelled *SUQ5*_{UAA} was obtained in a λ clone (APM16). The 1.1-kilobase *Bam*HI/*Hind*III subfragment of the *SUQ5*_{UAA} *B* fragment was transferred to pBR322 to produce the plasmid pPM16, which was used in the DNA sequencing. *b*, The map labelled *sup-RL1*⁺ refers to the original clone (APM15) of the *EcoRI C* fragment from the wild-type strain Y4A. A strategy almost identical to that described for *a* was used to re-clone the *C* fragment from the *SUP-RL1* *amber* strain DBD339 (obtained from D. Botstein). The tRNA^{Ser} gene on the *EcoRI C* fragment from Y4A was on a 2.5-kilobase *Bam*HI/*Hind*III subfragment, which was transferred to pBR322 to produce the plasmid pPM5—this was used in the DNA sequencing. A plasmid (pPM6) containing the 1.1-kilobase *Bam*HI/*EcoRI* subfragment of the *EcoRI C* fragment was used as a hybridization probe to isolate several independent λ clones that contained DBD339 *EcoRI C* fragments. From these recombinants, we chose a clone (APM10) that was missing the *TaqI* site found in the coding region of wild-type but not suppressor alleles of *SUP-RL1*. This clone, like all the DBD339 *C* fragment clones, contained a *C* variant that was 0.3 kilobases shorter than the Y4A *C* fragment. As shown in the map labelled *SUP-RL1*_{UAG}, the 0.3-kilobase deficiency maps between the 5' end of the tRNA^{Ser} coding region and the nearby *Hind*III site. The 2.2-kilobase *Bam*HI/*Hind*III subfragment that contains the tRNA^{Ser} coding region was transferred to pBR322 to produce the plasmid pPM11, which was used in the DNA sequencing.



tRNA^{Ser}-hybridizing fragments is unsurprising. However, the similarity in the amount of the missing DNA and the position of the deficiency relative to the tRNA coding regions in the two tRNA^{Ser} genes is quite striking. This impression is reinforced in the case of the two *SUQ5* fragments by DNA sequencing data that locate one of the homology/non-homology transitions at position -20 relative to the start of the region that codes for mature tRNA (data not shown). The beginning of the tRNA gene and the site of the deficiency, therefore, map within the same 0.4% of the *EcoRI B* fragment. The size variation is not a cloning artefact because it is readily observable by DNA-DNA hybridization to size-fractionated restriction digests of the pertinent yeast DNAs using the cloned *B* and *C* fragments as probes (data not shown).

Identity of the *SUQ5* and *SUP-RL1* clones

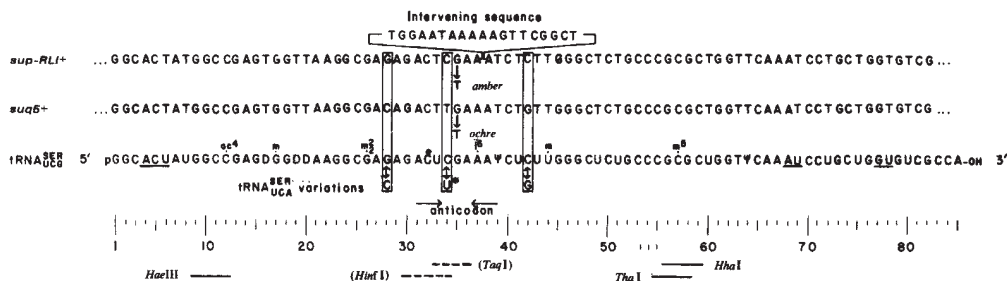
The tRNA coding regions were precisely located by searching for the cluster of restriction sites that are predicted from the tRNA sequence to occur within tRNA^{Ser} coding regions. An end-label, partial digestion technique was used to visualize directly the clusters of sites^{26,27}. DNA sequencing of all the

wild-type and mutant clones was carried out by the method of Maxam and Gilbert²⁸, starting at restriction sites in or near the tRNA^{Ser} coding regions. The results are summarized in Fig. 4 and confirm the assignments *B* = *SUQ5* and *C* = *SUP-RL1*: the tRNA^{Ser} gene on *B*, when cloned from a *SUQ5*_{UAA} strain, codes for an *ochre* suppressor, and that on *C*, when cloned from a *SUP-RL1*_{UAG} strain, codes for an *amber* suppressor. In both cases the only changes observed in the coding regions of the two mutant genes are the expected alterations at the sites coding for the second nucleotide in the tRNA's anticodon. The results are also consistent with the conclusion from RNA sequencing that the tRNA^{Ser} mixture contains at least two functional classes of tRNA^{Ser} (ref. 16). The *sup-RL1*⁺ gene codes for a tRNA whose anticodon is complementary to the serine codon UCG, while the *suq5*⁺ gene codes for a tRNA whose anticodon is complementary to UCA.

Comparisons of the *sup-RL1*⁺ and *suq5*⁺ sequences reflects a close but unusual relationship between these two genes. The regions that code for mature tRNA differ at only three sites, including the functionally critical one that codes for the tRNA's wobble position base. The *sup-RL1*⁺ gene, however, contains a

Fig. 4 The DNA sequences

of the regions of the *SUP-RL1* and *SUQ5* genes that code for mature tRNA and the *SUP-RL1* intervening sequence. Only the (+)-strand is shown (that is, the strand whose sequence corresponds to that of the tRNA). The three sites of variation between the two wild-type genes are boxed. The two dotted restriction sites (*Hinf*I and *Taq*I) occur in the *sup-RL1*⁺ but not the *suq5*⁺ gene. The *Taq*I site is lost in the *SUP-RL1*_{UAG} allele. The tRNA sequence has been corrected so that it is consistent with the DNA sequence. Positions in the corrected tRNA sequence which differ from the published sequences^{15,16} are underlined: (1) nucleotides 4-6 were misordered in the amino acid acceptor stem; (2) a complementary misordering occurred for nucleotides 77 and 78, which pair with nucleotides 4 and 5 in the mature tRNA structure; (3) the *SUP-RL1* gene is identical to that for *SUQ5* in the region that codes for positions 68 and 69, and the predicted RNA sequence is A-U. In previous work^{15,16}, a minor T1 oligonucleotide was observed that appeared to contain the sequence U-C in these positions. This 'U-C' oligonucleotide was present in molar amounts in the precursor to the *sup-RL1*⁺ gene product, and the 'A-U' variant was absent from this precursor¹⁶. Consequently, the U-C sequence was assigned to tRNA^{Ser}_{UCG} and the U-A sequence to the more abundant tRNA^{Ser}_{UCA} component, which we have now shown is partly a product of the *SUQ5* locus. The DNA sequence is not consistent with this interpretation and requires that both the *sup-RL1*⁺ and *suq5*⁺ gene products have the sequence AU. The C* at position 32 in the tRNA sequence is probably 3-methylcytosine (P.W.P., unpublished results), while the U* at position 34 is probably a derivative of 5-carboxymethyluridine (see text). The entire coding region sequence was determined for the *sup-RL1*⁺ gene (plasmid pPM5) and the *SUP-RL1*_{UAG} allele (pPM11), and also for the *suq5*⁺ gene (pPM15). In the case of the *SUQ5*_{UAA} allele, the sequence was determined only for positions +26 to +68. Considerable flanking sequence was also determined for three of the clones: *sup-RL1*⁺ (pPM5), from -67 to +119; *SUP-RL1*_{UAG} (pPM11), from -73 to -10 and -1 to +97; *suq5*⁺ (pPM15), from -100 to +119. Within the portions of the flanking regions where both pPM5 and pPM11 were sequenced, the sequences of the two clones were identical. Very little flanking sequence was determined for *SUQ5*_{UAA} (pPM16), but as discussed in the text, it was established that pPM15 and pPM16 are homologous from -7 to -20 and then diverge in sequence. The (+)-strand flanking sequences for *SUP-RL1* from -73 to +119, with asterisks at the site of the coding region, are as follows (slashes precede nucleotides -70, -60...+90, etc.):



C(-73)TT/GGATCTGTGA/AAAAACGCCT/AGAAACATGT/ATAAAACCTA/CCGCTTTAAA/CCATAATTTT/AAATCGAAAT(-1)/
 ***T(+83)TTAATT/TTTTTAAAT/AACATCGTTG/ATTAAAAACA(+119).

In the same format, the flanking sequences for *SUQ5* from -100 to +119 are as follows:

G(-100)TTATAAGGT/TGTTTCATAT/GTGTTTTATG/AACGTTTCAGG/ATGACGTATT/GTCATACTGA/CATATCTCAT/TTTGAGATAC/
 AACACTACCA/GATTATTTTG(-1)***T(+83)ATTATT/TTTTGAAAT/ATTTTCAAT/AACACTACATG(+119).

Table 1 Relationships between the yeast tRNA^{Ser} iso-acceptors that can translate the codons UCC, UCU, UCA and UCG

Iso-acceptor	Anticodon	Probable codon preferences	Apparent gene no.	Intervening sequence?	Corresponding suppressors
tRNA ^{Ser} _{UCG}	CGA	UCG	1	Yes	SUP-RL1 ^{UAG}
tRNA ^{Ser} _{UCA}	U*GA	UCA	3	No	SUQ5 ^{UAA}
tRNA ^{Ser} ₂	IGA	UCA(?), UCC, UCU	~11	No	None known

In addition to our results, we used other data on tRNA^{Ser}_{UCG} (refs 15, 16, 18 and 50), tRNA^{Ser}_{UCA} (refs 15, 16) and on tRNA^{Ser}₂ (refs 14, 17 and 34). On the basis of sequencing studies of tRNA^{Ser} species purified from brewer's yeast, a fourth iso-acceptor, tRNA^{Ser}₁, has been reported; it differs in sequence from tRNA^{Ser}₂ at three sites but has the same IGA anticodon¹⁴. This iso-acceptor is apparently absent from laboratory strains of *Saccharomyces cerevisiae*^{15,17,34}.

19-base pair intervening sequence but the *suq5*⁺ gene has none. The *sup-RL1*⁺ gene's intervening sequence, which was originally discovered by the RNA sequencing of a precursor to the mature tRNA¹⁶, occurs at a uniquely defined position on the gene's transcript, one nucleotide away from the 3' end of the anticodon. This positioning of the intervening sequence is highly, but perhaps not universally, conserved among tRNA genes^{7,29-33}.

The DNA sequences require minor revision of both published tRNA^{Ser}_{minor} sequences, as discussed in Fig. 4 legend. They also establish that the unidentified base present at position 32 in all the tRNA^{Ser}_{minor} species is a modified cytosine.

Outside their coding regions, the two genes display structural features that are common to many eukaryotic tRNA genes (see Fig. 4 legend). The CCA is not encoded, there is a presumed terminator tract of about 20 almost uninterrupted A·T pairs immediately after the coding region with a strong bias towards Ts in the (+)-strand and there is no obvious homology within the 5' noncoding regions despite the near identity of the coding sequences.

Discussion

Our observations of the DNA sequences that code for components of the yeast tRNA^{Ser}_{minor} mixture, combined with the results of previous studies, lead to a schematic overview (Table 1) of the 'UCN' family of genes that code for serine tRNAs recognizing the UCC, UCU, UCA and UCG codons. Most of these genes code for tRNA^{Ser}₂ (ref. 34). This species also accounts for by far the major portion of the serine acceptor capacity of yeast tRNA¹⁷. The two genes whose sequences we report (*SUP-RL1* coding for tRNA^{Ser}_{UCG} and *SUQ5* coding for tRNA^{Ser}_{UCA}) belong to a minor branch of the UCN family, which appears, on the basis of our RNA-DNA hybridization data, to contain four genes. The two sequenced cases differ from each other by only three nucleotides (4%) in the region coding for mature tRNA, while both differ by 11 nucleotides (13%) from the tRNA^{Ser}₂ genes. The 87% homology between tRNA^{Ser}₂ and the tRNA^{Ser}_{minor} species should not be taken as evidence of a particularly recent divergence between these two branches of the UCN family because yeast tRNA^{Ser}₂ even shares 78% homology with the rat liver tRNA^{Ser}₂ (ref. 35).

The special function of tRNAs with the U*GA anticodon (that is, the *suq5*⁺ product tRNA^{Ser}_{UCA}, and also the presumably similar or identical products of the less well-characterized tRNA^{Ser}_{minor} genes on *EcoRI* fragments *A* and *D*) is intriguing. The original wobble hypothesis allowed U·G pairings between a U in the first position of the anticodon and a G in the last position of the codon³⁶. However, unmodified Us are almost unknown in this position of RNAs and there is evidence that one of the effects of the ubiquitous modification of first position uridines is to restrict translational specificity³⁷. In the case of the

yeast tRNA^{Ser}_{minor} species with a U*GA anticodon, the U* is probably a derivative of 5-carboxymethyluridine as this is found in alkaline hydrolysates of tRNA^{Ser}_{minor} (P.W.P., unpublished results). Derivatives of 5-carboxymethyluridine have been found in the first positions of the anticodons for yeast tRNA^{Ser}₂³⁸, tRNA^{Ser}₃^{Arg} and tRNA^{Ser}₃^{Glu}, and in all three cases triplet binding studies indicate a strong preference for A over G in the last position of the corresponding codons³⁸⁻⁴⁰. In view of these precedents, the hypothesis that the U*GA anticodon is largely restricted to the translation of UCA is reasonable. This restriction would explain why *sup-RL1*⁺ is an essential gene because its gene product would presumably be the only tRNA^{Ser} that could efficiently translate UCG. The evolution of a special class of UCA-specific tRNAs suggests that the IGA anticodon of the major tRNA^{Ser}₂ species may be poorly suited to the translation of UCA. A hint that UCA codons lead to suboptimal translational efficiency in yeast is found in the DNA sequences of two genes that code for the highly abundant glycolytic enzyme glyceraldehyde-3-phosphate dehydrogenase: in 50 occurrences of serine codons, only UCU and UCC are used⁴¹.

Whatever the selective pressures that gave rise to this gene family, the close sequence relationship (96% homology) between tRNA^{Ser}_{UCG} and tRNA^{Ser}_{UCA} reflects an unusual evolutionary phenomenon: these genes have diverged functionally with the alteration of only three base pairs. Two of these alterations seem to be functionally neutral as their only consequence is a change from a G·C to C·G base pair in the anticodon stems of the tRNA products. The third alteration, however, affects the functionally critical site that codes for the wobble position base of the tRNA's anticodon. The presence of an intervening sequence in one of these genes (*SUP-RL1*) and its absence in the other (*SUQ5*) is intriguing. Like the two non-allelic rat insulin genes⁴², the yeast tRNA^{Ser}_{UCG} and tRNA^{Ser}_{UCA} genes represent a situation in which one gene in a closely related pair has either gained or lost an intervening sequence during an evolutionary period that was too short to allow substantial coding region divergence. These cases, as well as the existence of a strain-dependent pattern of intervening sequence occurrence in rRNA genes in the *Tetrahymena* micronucleus^{43,44} and yeast mitochondria^{45,46} cast doubt on the hypothesis that only extremely rare evolutionary events can result in the acquisition or loss of an intervening sequence from a functional eukaryotic gene^{47,48}.

Note added in proof: Two recent studies support the view of the tRNA^{Ser} gene family described here, and they provide important new details about the tRNA^{Ser}_{UCA} genes: a third suppressor of the *SUQ5* type, *SUP17*, has been discovered⁵¹ and the three suppressors *SUQ5*(= *SUP16*), *SUP17* and *SUP19* have been individually correlated with a set of three tRNA^{Ser}_{minor}-hybridizing *HindIII* fragments, which are the counterparts of our *EcoRI* fragments *A*, *B* and *D* (J. R. Broach, L. R. Friedman and F. Sherman, personal communication).

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LETTERS TO NATURE

Baryosynthesis and the origin of galaxies

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In the gravitational instability theory, the present structure in the Universe is envisaged to have evolved from small initial inhomogeneities which are of two generic types: isothermal and adiabatic. It has been shown that in the grand unified theory (GUT) for baryosynthesis primordial isothermal perturbations are incompatible with the standard Friedman–Robertson–Walker (FRW) universe. Here we discuss four non-standard models in which the two are compatible. Of these models two prove to be viable: (1) a universe which is initially dominated by large-scale inhomogeneous shear and (2) a universe which undergoes a de Sitter phase during the GUT phase transition. We also show how to deduce new constraints on chaotic behaviour near the cosmological singularity.

Two outstanding fundamental problems in cosmology are the origin of matter and the origin of structure—stars, galaxies and clusters. Although the laws of physics are very nearly matter–antimatter symmetrical, the Universe seems to possess an overt asymmetry and contains a negligible amount of antimatter¹. The average ratio of baryons to 3K microwave photons² is $10^{-10 \pm 1}$; the baryon-to-specific entropy ratio (kn_B/s), which remains constant when baryon number n_B is conserved and the expansion is isentropic, combines these two observations², $B \equiv kn_B/s = 10^{-11 \pm 1}$. In the gravitational instability picture (reviewed in refs 3, 36), the present large-scale structure developed from small-density inhomogeneities which existed earlier but the origin of these fluctuations is a key problem. These perturbations are of two generic types: adiabatic, in which both the matter and radiation participate and which are isentropic, $\delta B = 0$, and isothermal, which during the radiation-dominated epoch are simply spatial fluctuations in B . During the radiation epoch adiabatic perturbations on scales larger than the horizon ($|x| \geq t$) are gravitationally unstable and amplify^{3,36} in co-moving proper time, $\delta_A \equiv \delta\rho/\rho \propto t$, while isothermal perturbations, $\delta_I \equiv \delta B/B$, remain constant. In each case, when the matter and radiation decouple ($t \sim 10^{13}$ s) density perturbations on scales greater than the Jeans mass at decoupling are Jeans unstable and grow to produce the present structure. (The possibility of massive neutrinos modifies this picture, see ref. 4; but for present purposes these changes are not important.) There is

no compelling evidence for the initial perturbations to be of any one type. However, if the initial perturbations were adiabatic, then variations in the microwave background⁵ of $O(10^{-4})$ should be observed on scales $\geq O(30'')$. The present upper limits on such variations are very close to the expected variations⁵. The details of galaxy formation depend critically on which of these types of primordial perturbations were present.

The solution to one of these fundamental problems seems to preclude, or at least exacerbate, the solution to the other. For example, first, theories^{6,7} in which the microwave background is of recent origin (cold universe models) have been advocated to explain the origin of structure; however, they offer no clue as to why the Universe contains only matter whilst the laws of physics are nearly matter–antimatter asymmetrical. Second, great progress has been made recently in understanding the origin of $kn_B/s \approx 10^{-11}$ within the context of the standard hot big-bang model⁸. It has been suggested^{10–15} that the out-of-equilibrium decay of super massive ($\approx 10^{14}$ GeV) gauge or Higgs bosons which are predicted by GUTs, and whose interactions violate B, C and CP, can lead to the production of a baryon excess of the required magnitude ($B \approx 10^{-11}$). Detailed numerical calculations^{16,17} show that this type of explanation is predictive and can work. However, in the standard FRW model of the hot universe the GUTs preclude the production of isothermal perturbations during baryosynthesis—which could be a serious limitation in attempts to explain the formation of large-scale structure in the universe¹⁸.

We now examine four non-standard models in which it is possible to have both baryosynthesis and isothermal perturbations. The first two have been suggested elsewhere and involve curvature fluctuations⁷ and primordial black holes^{18–20}. In the third the dynamics of the universe are initially dominated by large-scale inhomogeneous shear, and in the fourth the universe undergoes a de Sitter phase due to a first-order phase transition^{21–24}.

We first need to explain why the GUTs scenario for baryosynthesis excludes the existence of isothermal perturbations in the standard hot big-bang model. Detailed calculations^{16,17} show that the baryon asymmetry produced by superheavy boson decay is given by

$$B = 10^{-2} \varepsilon f(\alpha, K) \quad (1)$$

where ε measures the CP violation in the superheavy system ($\equiv$ mean net baryon number produced by the decay of a super heavy boson–antiboson pair), α is the coupling strength of the superheavy, and $K = \Gamma_D/H$ for $T = M$ (Γ_D is the decay rate of the superheavy boson, M is the mass of the superheavy boson, and $H \equiv \dot{R}/R$ is the expansion rate of the universe) is a measure of the deviation from local thermal equilibrium. In the FRW universe²⁵ $H = (8\pi G\rho/3)^{1/2}$ and $\rho = g_*(T)(\pi^2/30)T^4$, so that

$$H^2 = \frac{8\pi G\rho}{3} g_* \frac{\pi^2}{30} T^4 \equiv AT^4 \quad (2)$$

where $g_*(T)$ counts the relativistic degrees of freedom ($g_* = \sum_{\text{bosons}} g + 7/8 \sum_{\text{fermions}} g$). $\Gamma_D = c\alpha M$, where $c \sim 0(1)$ and depends on the GUT. Therefore,

$$K = c \left(\frac{4\pi^3}{45} \right)^{-1/2} g_*(M)^{-1/2} \alpha (GM)^{-1/2} \quad (3)$$

so that B only depends on particle physics parameters and constants, and can have no spatial variation. In minimal SU(5) $K = 3 \times 10^{17} \alpha \text{ GeV}/M$. For $K \ll 1$, $\Gamma_D \ll H$ when $T \approx M$ and so superheavy bosons cannot diminish in number and remain overabundant (relative to their equilibrium abundance) for $T \lesssim M$. When they eventually do decay, ($T \ll M$), they produce a baryon excess. In this case, $f(\alpha, K) \approx 1$ and the maximum asymmetry arises. For $K \gg 1$, $\Gamma_D \gg H$ and decays do occur when $T \approx M$; an equilibrium abundance of superheavy bosons is maintained to some extent, and a lesser asymmetry evolves. For $1 \lesssim K \lesssim 30$, numerical results¹⁷ reveal

$$f(\alpha, K) \approx K^{-1.3} \quad (4)$$

(essentially independent of α). From equations (1), (3) and (4), to produce $\delta_1 \neq 0$, one must either modify the time-temperature relationship (2) in a spatially dependent way, have a spatially dependent ϵ , or modify the GUTs scenario.

If the GUTs scenario for baryosynthesis is discarded, then arbitrary initial fluctuations in B can be specified. In the GUTs picture it is still possible to specify an initial $B(\mathbf{x})$; however, in most instances the initial $B(\mathbf{x})$ is destroyed by baryon nonconserving reactions¹⁷. If the initial $B(\mathbf{x})$ is associated with some quantity conserved by the GUT, then the initial fluctuations will survive—but this is not a very appealing possibility.

Neither the existence nor the survival of adiabatic inhomogeneities ($\delta_A \neq 0$) in the early universe depend on the GUTs. However, it might be possible to demonstrate that if adiabatic fluctuations are present in the early universe when baryosynthesis is occurring, then a spatial variation in K ($\sim$ baryosynthesis efficiency) must occur. A large-scale ($|\mathbf{x}| > t$) adiabatic inhomogeneity is equivalent to a gradient in the spatial three-curvature of space-time ($\delta_A \sim \delta^{(3)}R$). If we suppose this curvature inhomogeneity to be spherically symmetric, then, in accord with Birkhoff's theorem²⁵, it will behave as an independent Friedmann universe embedded in a flat background. The temperature-time adiabat of the spatially flat background will be given by equation (2), and that of the inhomogeneity by

$$\tilde{H}^2 = A\tilde{T}^4 - C\tilde{T}^2 \quad (5)$$

where $\tilde{}$ denotes quantities parameterizing the inhomogeneity and C is the curvature parameter. The efficiency of baryosynthesis in the two regions can be computed from the parameters K and $\tilde{K}$:

$$\tilde{K}/K = \left(1 - \frac{C}{AM^2} \right)^{-1/2} \quad (6)$$

According to equation (4) we see that when the amplitude of the inhomogeneity is small and $K \gg 1$, the ratio of specific entropies in the regions of differing curvature departs from unity

$$\tilde{B}/B \approx 1 - 0.65 \frac{C}{AM^2} \quad (7)$$

In general $C(\mathbf{x})$ is some fairly arbitrary function of space (or mass scale). Where $C > 0$, the overdense regions expand more slowly than the flat ($C = 0$) background, equilibrium is more easily maintained, and consequently a smaller baryon number is generated than in the background. Note that this inhomogeneous distribution of baryons occurs when $K \gg 1$, $M \lesssim 10^{16}$ GeV for gauge bosons and $M \lesssim 3 \times 10^{13}$ GeV for Higgs bosons, and also that regions of radiation excess ($C > 0$) finally emerge with a baryon deficit. Note that $f(\alpha, K)$ has a slight K -dependence for $K < 1$, so that small variations in B will arise even if $K < 1$.

Although this argument indicates that, in principle, adiabatic modes generate isothermal ones of similar amplitude and spec-

trum at $t_{\text{GUT}} \sim 10^{-35}$ s, in practice these isothermal modes will be physically irrelevant to the origin of galaxies and clusters. During the subsequent period of universal expansion between $t \sim 10^{-35}$ s and the end of the radiation-dominated era, $t \sim 10^{12}$ s, the isothermal modes will not grow, whilst the accompanying adiabatic modes will grow, $\delta_A \propto t$, until they enter the horizon. By the epoch of galaxy formation the amplitude of any isothermal modes will be utterly negligible compared with any adiabatic ones that were of similar amplitude at t_{GUT} .

If curvature fluctuations have large amplitude ($C/AM^2 \sim 1$) then equation (5) indicates that $\tilde{H}$ may fall to zero. If this occurs when $|\mathbf{x}| \sim t$ then black hole formation is inevitable. It has been suggested that this creates another means of producing isothermal fluctuations^{18,19}:

Black holes smaller than $m_* \sim 4 \times 10^{-2} m_p^2 M^{-1}$ will have a Bekenstein-Hawking temperature, T_{BH} , in excess of $M(m_p = G^{-1/2} \approx 1.2 \times 10^{19} \text{ GeV})$. When they evaporate, a significant portion of their mass is radiated as superheavy boson ($X\bar{X}$) pairs. The $X\bar{X}$ ejecta decay out of equilibrium and generate an inhomogeneous distribution of baryons through their baryon-asymmetric decays. The amplitude and spectrum of the final baryon inhomogeneity are simply related to those of the adiabatic perturbations that collapsed into black holes. Again, an isothermal spectrum can be generated but the subsequent growth of the adiabatic perturbations associated with the formation and explosion of the holes will render the isothermal modes completely insignificant in the late stages of expansion.

Another type of inhomogeneity could be present in the earliest stages of the universe ($t \lesssim 10^{-35}$ s); one which does not suffer from the above-mentioned difficulties, and which, in the course of expansion will decay away adiabatically as t^{-1} . Suppose the early universe were to contain an inhomogeneous distribution of shear (gravitational wave) anisotropy, that is, at every point $\mathbf{x}$ the three orthogonal expansion rates would differ when measured at the same proper time from the initial singularity. When the shear inhomogeneity is large scale ($|\mathbf{x}| > t$), then a good description of the behaviour of the whole space-time is given by the velocity-dominated approximation²⁶⁻²⁸. If we assume the singularity is simultaneous in space at $t = 0$, then the temperature-time relationship is a weighted combination of Kasner and Friedmann behaviours,

$$\rho = (g_* \pi^2 / 30) T^4 = \frac{3}{32\pi G} t^{-4/3} (t + t_*(\mathbf{x}))^{-2/3} \quad (8)$$

where the arbitrary spatial function $t_*(\mathbf{x})$ indicates the time when the expansion at $\mathbf{x}$ passes from being anisotropic to roughly isotropic (t_* is directly proportional to the gravitational wave energy at $\mathbf{x}$). Equation (8) describes a universe that begins ($t \ll t_*$) as an inhomogeneous Kasner universe but expands to approach a flat Friedmann model to a better and better approximation in the course of time, $t \gg t_*$ (overbar here denotes the mean value of the spatial function $t_*(\mathbf{x})$). It is known that exact solutions to the Einstein equations exist which possess inhomogeneous shear anisotropy of this form. However, it is not known whether this behaviour is generic to cosmological solutions of the Einstein equations or whether curvature fluctuations must generically co-exist with shear fluctuations.

Suppose the shear inhomogeneity is large at the time of baryosynthesis, $t_{\text{GUT}} \ll t_*$, then the shear distribution creates a spatial variation in K . We find

$$K(\mathbf{x}) \propto t_*^{-1/2}(\mathbf{x}) \quad (9)$$

If K exceeds unity, baryosynthesis will reflect this inhomogeneity, and create isothermal fluctuations with

$$\delta_1 = (2/3) \delta M^{-\alpha} \quad (10)$$

where $t_*(\mathbf{x}) = \bar{t}_*(1 + \delta M^{-\alpha})$ describes the spectrum of inhomogeneous shear on the mass (length) scale $M(|\mathbf{x}|)$. Although the dependence $B \propto K^{-1.3}$ strictly only applies in the isotropic case, we have assumed that it is also a reasonable description in the anisotropic case. When the amplitude of the shear inhomogeneity is small, $t_{\text{GUT}} \gg \bar{t}_*$ a similar trend is obtained

$$K(\mathbf{x}) \propto 1 - \frac{1}{9} [t_*(\mathbf{x})/t_{\text{GUT}}]^2 \quad (11)$$

$$\delta_1 \approx 0.3 \delta (t_*/t_{\text{GUT}})^2 M^{-\alpha}$$

(if $K < 1$ lesser fluctuations arise).

The shear inhomogeneity will subsequently decay away in accord with equation (8), but the baryon fluctuations will remain to constitute an isothermal spectrum of density inhomogeneities in the later stages of the expansion. It is possible to 'explain' any desired isothermal spectrum by a suitable choice of the gravitational wave energy density $t_*(\mathbf{x})$ —a situation no less arbitrary than in all other models where the isothermal spectrum is given directly by initial conditions. (Note that a spectrum of primordial rotational inhomogeneities could generate baryon inhomogeneity but the rotational perturbations will subsequently grow in amplitude relative to the radiation density as $\omega^2/\rho \propto t$ until the end of the radiation era; ω is the angular velocity. If they were large enough to generate significant primordial baryon number fluctuations they would now be too large to be compatible with present microwave background isotropy measurements.)

There is another interesting consequence of this generation process. Suppose the very early ($t \leq 10^{-35}$ s) universe were chaotic in structure^{29,30}, dominated by inhomogeneous and anisotropic motions as described by a relation of the form of equation (8) where $t_*(\mathbf{x}) \gg t$. In the absence of the GUTs scenario, the chaos would decay away $\propto t^{-1}$ in the course of time and this information concerning the very earliest stages of the universe would be unavailable. However, if B evolves through the GUTs picture, then the chaotic dynamics before time t_* are imprinted in the baryon distribution and this information is not redshifted away. In particular, shear fluctuations of large amplitude ($\nabla_x t_* \gg 1$) on a scale $\mathbf{x}$ would produce baryon inhomogeneities of such large contrast ($\delta_1 \gg 1$) that at the end of the radiation era, when freed from the radiation background, they would form a profusion of supermassive black holes on all scales $\mathbf{x}$ exceeding the Jeans mass at $t_{\text{rec}} \sim 10^5 - 10^6 M_\odot$. The strong observational limits^{31,32} on the presence of large black holes indicate that the universe was not dominated by very large-amplitude gravitational wave inhomogeneities at $t \sim 10^{-35}$ s. If small-amplitude isothermal modes were to arise from shear inhomogeneities, then the geometry of the baryon distribution on very large scales (~ 100 Mpc) might be anticipated to reflect the ellipsoidal configurations of the gravitational wave modes. Pronounced flattening of very large-scale structures might be expected which would reflect the dynamics of the universe at t_{GUT} rather than local gravitational instabilities which only generate deviations from spherical symmetry in much smaller structures that undergo collapse.

Finally, there is another way to produce primordial isothermal fluctuations within the GUTs scenario. From equation (1), B depends only on α , K and ε . We have already discussed the consequences of producing spatial variations in K . We will now consider how spatial variations in ε might give rise to isothermal perturbations. The CP violation ε is one of two generic types: (1) hard, in which CP-violating terms appear explicitly in the lagrangian, and (2) soft, where the violation is a consequence of spontaneous symmetry breaking. In the former, ε is constant throughout the universe, and so could not give rise to $\delta_1 \neq 0$. In the latter, ε depends on phase angles 'chosen' by the symmetry breaking. These should be uncorrelated over dimensions larger than the particle horizon at the time of symmetry breaking ($t \sim t_{\text{GUT}}$). (The analogue in ferromagnetism is the different directions of magnetization in independent domains of a ferromagnet.)

In most models incorporating soft CP violation, the sign of ε varies from domain to domain, so that the universe is on the whole matter-antimatter symmetrical (up to purely statistical fluctuations)³³. Yanagida and Yoshimura³⁴ have suggested that only the magnitude (and not the sign) of ε may vary. We might call this 'semi-soft' CP violation. (One can create an *ad hoc* model by considering the possibility of having both hard and soft

CP violation, so that $\varepsilon = \varepsilon_H + \varepsilon_s$.) In this case we would have

$$\varepsilon(\mathbf{x}) = \varepsilon_0 [1 + \delta \text{sgn}(\cos \theta(\mathbf{x}))] \quad (12)$$

where $\theta(\mathbf{x})$ varies randomly on scales larger than a domain. So we expect baryosynthesis to create a Poisson spectrum of isothermal perturbations,

$$\delta_1 \approx (M/M_D)^{-1/2} \quad (13)$$

where M_D is the baryon mass contained in a domain at t_{GUT} . The difficulty is that a domain can be no larger than a region which can internally communicate. In the FRW model this is just the volume within the horizon $\approx (4\pi/3) \times (2t)^3$, so that the number of baryons contained in this volume is

$$N_B = (kn_B/s)(s/k)(32\pi/3)t^3$$

$$= 10^{-11 \pm 1} (5/\pi^3)^{1/2} g_*^{-1/2} (T)(m_p/T)^3 \quad (14)$$

For $T \approx 10^{15}$ GeV and $g_* \sim 100$, $N_B \approx 10^{\pm 1} \approx 10^{-57} M_\odot$. The isothermal fluctuations on the scales of interest ($M \geq 10^5 M_\odot$) are $\leq 0(10^{-31})$ which is too small to be of any significance.

However, it has been suggested²¹⁻²⁴ that the GUT phase transition may be first order, and that if the transition is slow enough, then the universe goes through a de Sitter phase. During this period the horizon grows exponentially, $d_H \propto \exp(t/\tau)$. If the transition takes $\sim 100 t_{\text{GUT}}$, then M_D could easily exceed $10^5 M_\odot$. If this were the case, the semi-soft scenario could provide the required spectrum of isothermal perturbations. (Numerical simulations have demonstrated that $\delta_1 \approx (M/10^6 M_\odot)^{1/2}$ can reproduce the present structure^{3,36}.) There is no way at present to calculate whether or not the de Sitter phase should occur or how long its duration would be if it did. In addition there seem to be difficulties associated with the transition back to a FRW universe from the de Sitter phase²¹, although Eardley and Press (personal communication) may have found a solution to this difficulty.

Although in the standard FRW model baryosynthesis and isothermal protogalaxies are mutually incompatible, we have suggested two scenarios in which they are both allowed. In the first, the universe must initially be dominated by large-scale inhomogeneous shear, and the superheavy boson responsible for baryosynthesis should be less massive than $\approx 3 \times 10^{17} \alpha$ GeV. The spectrum of isothermal perturbations mirrors that of the inhomogeneous shear. In the second, the CP violation ε must be semi-soft and the universe must undergo a de Sitter phase. A Poisson spectrum of isothermal fluctuations is produced.

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Impact fusion and the field emission projectile

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A conceivable approach to the problem of achieving controlled thermonuclear power is by impact fusion¹⁻³. Small projectiles (macrons⁴), of mass ~ 0.1 g and of appropriate material and design, are accelerated to a velocity $v \sim 10^8$ cm s⁻¹; they then collide with a deuterium-tritium target, and their kinetic energy is abruptly converted into thermal energy (~ 10 keV per nucleon, temperature $\sim 10^8$ K) that is inertially confined in a shocked region. Preliminary studies^{1,2} in the early 1960s showed that in these conditions the released nuclear energy exceeds the initial kinetic energy. Despite its attractiveness, relatively little research has been devoted to the development of impact fusion as a power-producing technology, and only recently⁵ have serious attempts been made to evaluate its importance compared with the more familiar proposed methods of magnetic and inertial confinement. The principal block to impact fusion is the development of accelerators capable of accelerating macrons to velocities two orders of magnitude greater than can be attained by existing methods. It is shown here, using general arguments, that the minimum length of such macron accelerators must exceed 1 km. A possible way of achieving the required high velocity is to accelerate, in a travelling electric field, macrons that are self-charged by electron field emission.

With a uniform acceleration $\dot{v}$, a nonrelativistic accelerator has a length

$$x = v^2/2\dot{v} \quad (1)$$

The differential or tidal stresses caused by an accelerating force F acting on a projectile of characteristic size L , are $\sim F/L^2$. If these stresses are not to exceed the yield stress s of the projectile, then the minimum length of the accelerator is

$$x_{\min} \sim \rho L b^2/s \quad (2)$$

for a projectile of density ρ and mass $\sim \rho L^3$. The criterion¹ for a net energy gain in impact fusion is approximately $\rho L > 0.1$ g cm⁻³. With a reasonable value for the yield stress, of $s \sim 10^{10}$ dyn cm⁻², it is found that the minimum accelerator length is $x_{\min} \sim 1$ km. In practice, the actual minimum length might be ~ 10 km. This result is reasonably general and applies to electrostatic, magnetic (for example, rail guns⁶), travelling electromagnetic wave, beam-pushing⁷, and laser ablation^{4,7} accelerators. A few years ago an accelerator length of 10 km seemed prohibitive and impact fusion was hence deemed to be of academic interest only. But existing and proposed accelerators of subatomic particles are now on this scale⁸, and because macron accelerators might conceivably be less costly, impact fusion has become a realistic possibility.

In spite of various problems (such as joule dissipation), it is widely thought that magnetic field acceleration is superior to electrostatic or travelling electric wave acceleration⁷. I shall

attempt to show that electric field acceleration of self-charged macrons is capable of attaining the velocities required for impact fusion.

Consider first a conducting sphere of radius r , polarized in an electric field E , and having a net positive charge Q . The surface charge density is

$$\sigma = (Q + 3r^2 E \cos \theta)/4\pi r^2 \quad (3)$$

where $\theta = 0$ lies in the direction of E . Imagine now a fine whisker of length h , small compared with r , protruding from the rear ($\theta = \pi$) of the sphere. The field strength at the tip of the whisker is

$$E_{\text{tip}} \sim -E(h/b)^2(1 - Q/Q_0) \quad (4)$$

where $Q_0 = 3r^2 E$, and b is the radius of curvature at the tip of the whisker. With $E \sim 10^5$ V cm⁻¹, $h/b \sim 10^3$, the field emission current⁹ is sufficient to create a charge $Q \sim Q_0$ on a time scale of 10^{-3} s. The acceleration is hence $\dot{v} = 9E^2/4\pi\rho r$, and the accelerator length is

$$x \sim \rho r v^2/E^2 \sim x_{\min} s/E^2 \quad (5)$$

where $x_{\min}$ is given by equation (2) with $L = r$. The maximum field intensity (at which disruption occurs) is $E_{\max} \sim s^{1/2}$, roughly equal to 3×10^7 V cm⁻¹. Usually, in practical applications we have $E \ll E_{\max}$, and therefore in this case the accelerator is exceedingly long.

Slightly better results are obtained when the trailing whisker has a length $h > r$. In this case the positive charge acquired by field emission is $Q_0 \sim rhE$, and the field strength necessary for maximum acceleration is hence $E_{\max} \sim (rs/h)^{1/2}$.

The above examples illustrate the proposed method, and show that the acceleration of spherical bodies charged by field emission is not very promising. The most striking results are obtained when we consider needle-shaped macrons. A prolate spheroidal body of $a \gg b$ (where a is the semi-major axis parallel to the applied electric field and b is the semi-minor axis), which is polarized and has a net positive charge Q , has an electric field at the trailing tip of

$$E_{\text{tip}} = (Q - Q_0)/b^2 \quad (6)$$

where

$$Q_0 = a^2 E / \left(\ln 2 \frac{a}{b} - 1 \right) \quad (7)$$

With a value of b sufficiently small, copious field emission of electrons occurs, and the positive charge Q tends to the value Q_0 . The attained charge is about the same in value as that on a sphere of $r = a$, or

$$(Q_0/M)_{\text{ellipsoid}} \sim (a/b)^{4/3} (Q_0/M)_{\text{sphere}} \quad (8)$$

and the charge-to-mass ratio is increased by $(a/b)^{4/3}$ compared with a sphere of similar mass and density. The accelerator length in this case is

$$x = 2\pi b^2 \rho v^2 \left(\ln 2 \frac{a}{b} - 1 \right) / 3aE^2 \quad (9)$$

The maximum possible applied electric field (at which disruption occurs) is found to be

$$E_{\max} \sim (b/a)s^{1/2} \quad (10)$$

thus yielding a minimum accelerator length in accord with equation (2) with $L \sim a$. Hence, when $a/b \sim 10^3$, only modest electric fields of $E \sim 10^4$ V cm⁻¹ are required in a travelling wave linear accelerator of length 10 km. If the tensile strength of the whisker projectile could be as great as $s \sim 10^{12}$ dyn cm⁻², then stronger electric fields would offer the prospect of accelerators only hundreds of metres in length.

Field-emitting macrons have the following advantages: they are self-charging; large positive charges are in principle obtain-

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able; the net positive charge is concentrated towards the fore end and the macron is stably oriented; and the charge-to-mass ratio is predetermined thus allowing for synchronous acceleration in a travelling wave accelerator.

In general, macrons must fulfil two requirements: they must have a composition and configuration that allow optimum acceleration, and must have a configuration that yields a high temperature and density on impact with the target. Field-emitting macrons, which are needlelike in shape, do not necessarily have a configurational interaction with the target that allows for efficient use of their kinetic energy. For a needle-like macron the fusion criterion $\rho L \geq 0.1 \text{ g cm}^{-2}$ applies with $b \leq L < a$, and a spherical macron having the same mass is undoubtedly superior¹. We have therefore an apparent conflict between these two requirements. It may be possible, by means of an intense magnetic field of $B \sim s^{1/2} \sim 10^5 \text{ G}$, to arrange for a change in configuration before impact with the target. Thus after acceleration, and before impact, the needle-like macron enters a region in which magnetic stresses cause it to collapse into a spherical body of radius $(ab^2)^{1/3}$.

The development of accelerators capable of accelerating macrons to high velocity will no doubt open up an entirely new field of 'macron physics'. Not only will it then be possible to study condensed matter at high temperatures, and perhaps achieve a controlled fusion technology, but with needle-like picogramme macrons it should also be possible to study relativistic effects ($v \sim c$) in macroscopic bodies.

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British shore platforms and ice-sheets

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During the past few years views about the relative ages and modes of formation of fossil rock platforms and cliffs in Scotland have changed considerably¹⁻³. I suggest further changes here and present a model of rock-platform relationships which involves an interglacial set of platforms, a glacial set, and a late-glacial feature. Interpretations of the glaciation of the Outer Hebrides have recently been completely revised: this revision has major implications for the glaciation of the Inner Hebrides which, in turn, has wider implications.

It was accepted for a century that the last ice-sheet from the Scottish mainland crossed the Outer Hebrides, except for the mountain area of Harris, where it was confluent with local ice. It is now known that almost the whole of the Outer Hebrides was last covered by an independent ice-mass, which flowed towards the east and south-east over most of the island chain. The evidence for this flow comprises ice-moulded landforms, striae, and directions of transport of erratics from sources in the islands^{4,5}. This means that the Scottish Highland ice-sheet terminated to the east of the Outer Hebrides. A stable ice margin in the mostly deep waters of the Minches and Sea of the

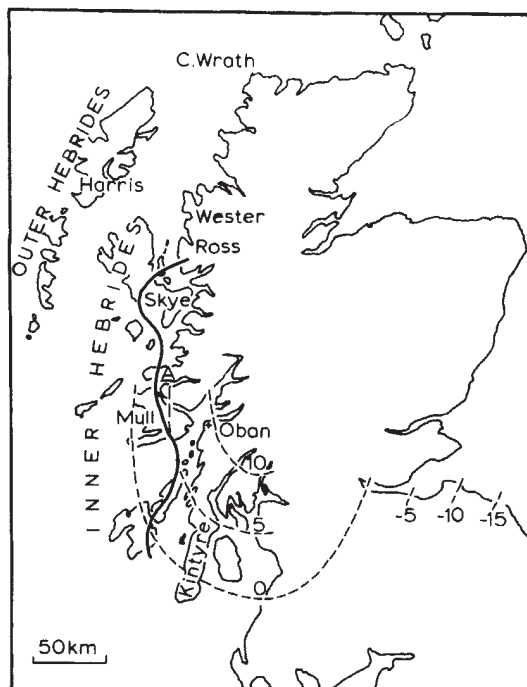


Fig. 1 Isobases for the Main Lateglacial Shoreline (after ref. 2) and eastern limit of high rock platforms as known at present (solid line). A, Ardnamurchan.

Hebrides seems improbable. Relative stability of the Highland ice margin amidst the Inner Hebrides, where much of the terminal zone would have been land-based, seems more likely. One may envisage the ice advancing into the sea to the west from time to time owing to climatic deteriorations, these advances being followed by rapid retreat through calving during climatic ameliorations.

Growth of an ice-mass on the Outer Hebrides presents problems because erratics derived from sources outside the islands are present and because much of the area is rather low ground. I have therefore suggested⁶ that the islands were covered by the last Highland ice-sheet (except for the mountains of Harris and surrounding ground). Subsequently, owing to calving, the major portion of the Highland ice-sheet was separated from ice on the Outer Hebrides. The situation described above should then have applied.

Shorelines cut in rock at reported altitudes of 18–51 m (mostly approximate and here converted to OD where necessary) are extremely well developed along many coasts in the Inner Hebrides. They also occur on the mainland coast in parts of Ardnamurchan and Wester Ross (Fig. 1)⁷⁻⁹. Some platform fragments are only 100–200 m wide but others are much broader, ranging up to 1 km. The backing cliffs are sometimes small, but others are tens of metres high, with a reported maximum of 90 m. Some platform fragments are striated and ice-moulded and some are overlain by till. This evidence of glaciation has led to the features being interpreted as formed during interglacials.

The interglacial interpretation encounters difficulties. (1) The required periods of prolonged interglacial sea-level stability are not recorded in the curves based on evidence from deep-sea cores¹⁰. (2) Because the platforms are much higher than platforms in England, Wales and Ireland that are regarded as interglacial, significant Quaternary uplift in the Inner Hebrides (unconnected with glacio-isostatic uplift) has been invoked. There is no independent evidence for such uplift. (3) 'High' platforms and cliffs are pronounced features in the Inner Hebrides and parts of the mainland west (and north) of the line shown in Fig. 1, but have not been reported east of the line.

Although glacial erosion can readily explain modification or removal of some platform fragments, it cannot alone account for this pronounced contrast.

In some limited areas high-platform fragments at quite different altitudes show clearly that more than one period of erosion is involved^{8,11,12}. Despite this evidence the fragments have usually been discussed as if they constitute a single feature called 'the preglacial platform' or 'the interglacial platform'⁷⁻⁹. This has produced a simple error in reasoning: even though some platform fragments carry no evidence of glaciation it has led to the assumption that they have, nevertheless, been glaciated. Having thus assigned all rock-cut features to a 'preglacial' age, it has then been inferred that late-glacial coastal erosion 'scarcely affected solid rock' being 'restricted to the removal of older drift deposits' (ref. 11, p. 21). No convincing evidence of glaciation has been found on most platform fragments in Mull and northeastern Skye^{8,13}. In Wester Ross some platform fragments form part of a late-glacial shoreline that elsewhere is represented by depositional forms (raised beaches and shingle ridges). Thus some platforms are not interglacial. Glaciated platforms also occur in Wester Ross but they are above the late-glacial marine limit^{14,15}.

It has been suggested that all the high-platform fragments in Wester Ross were formed by periglacial shore erosion¹⁵. It has also been argued that the 'high' platforms of western Scotland as a whole were produced during build-up of the last Scottish ice-sheet³. I now propose that the high-platform fragments represent a series of platforms produced during glacials. Frost riving would have been important, its effectiveness having been facilitated by removal of the resultant debris by waves and by repeated wetting in the intertidal zone during the period of each year when sea ice was absent. In sheltered areas (such as northeastern Skye and Wester Ross) frost riving would have been of major importance. In more exposed areas, where platforms are most extensive (for example, parts of western Mull), strong wave action would have operated in conjunction with frost action.

It is proposed that the altitudes of the features reflect glacio-isostatic uplift. The apparent absence of high platforms east of the line shown in Fig. 1 is attributed mainly to the presence of the Highland ice-sheet and of ice nourished on the high ground of eastern Mull and south-central Skye. The line (or one approximating to it) is interpreted as roughly representing the relatively stable position of the margin of this ice-mass, as mentioned above. I suggest that the platforms and cliffs were shaped and reshaped during successive glacials. Many glaciated platform fragments may be attributed to formation during ice-sheet advance³ or readvance, when the relationships between eustatic sea-level and glacio-isostatic depression of the land differed from those during the last deglaciation of the localities in which they occur.

It has been inferred from deep-sea core evidence that major buildup of the North American and Scandinavian ice-sheets occurred at ~75 kyr ago¹⁶. If major buildup of the last Scottish ice-sheet also occurred at this time³, there is likely to have been a long period during the last-glacial when 'high' platforms and cliffs could have been formed in western Scotland.

Along parts of the coastline of western Scotland there is a lower platform and cliff. The platform is typically 20–30 m wide, but sometimes exceeds 150 m, and the cliff is up to 15 m high. These features, often continuous for many kilometres, were

initially interpreted as produced during the Holocene⁸ but were subsequently regarded as interglacial^{9,11,17,18}. I suggested the features were formed during the Loch Lomond Stadial (approximately equivalent to the Younger Dryas Stadial) and the later part of the preceding interstadial by frost action accompanied by limited wave action¹. This view was doubted¹⁹ but has been justified by detailed studies^{20,21} which show that the shoreline (Main Lateglacial Shoreline) reaches ~11 m north-east of Oban, whence it slopes down towards west, southwest and south (Fig. 1). The feature has not been glaciated outside the limit of the Loch Lomond Advance but inside the limit only the cliff (or part of the cliff) is normally visible.

The Main Lateglacial Shoreline is also identified in south-eastern Scotland, where it is buried by later deposits or submerged by the sea. Its eastward slope has been traced from slightly above OD to -18 m (Fig. 1)²²⁻²⁴. Owing to less resistant rocks than in western Scotland, the rock platform is often hundreds of metres wide; in drift it may attain 2 km. Because late-glacial marine deposits are truncated by the plane of erosion and as deposits older than ~9,600 BP overlie it, a late-glacial age is strongly suggested. Such considerable planation requires a stable or more probably, a slowly transgressing sea. Stability followed by slow transgression during a period of 1,000–1,500 yr has been inferred, at the appropriate altitudes, from independent studies of marine fauna from sites in western Scotland^{25,26}.

Intertidal rock-platform fragments have been reported from many places in the Inner Hebrides and on the west and east coasts of the Scottish mainland. They are normally far less impressive features than those described above. Many of the fragments are ice-moulded or bear striae and some pass inland beneath cliffs of till. Interglacial formation is widely agreed. It has been recently proposed that intertidal platform fragments in part of the Inner Hebrides are parts of a single horizontal platform² but this view needs qualifying, first because intertidal platforms are obvious features owing to removal of any overlying deposits. They may therefore be a biased sample of a larger population. Second, where intertidal platforms, rising landwards, pass beneath till, the latter normally survives because it is above the reach of effective wave action. Because it is rarely possible to measure the inner edge of these higher, till-covered platforms the sample is again biased. Finally, in almost the whole area where detailed measurements have been made of the inner edge of intertidal fragments, the platform associated with the Main Lateglacial Shoreline also exists: in a considerable part of the area the latter is within a few metres of present sea-level. Consequently it is highly unlikely that interglacial platforms will have survived in the altitudinal zone affected by the later erosion. In this context it is very significant that in Kintyre, as the Main Lateglacial Shoreline descends to and passes below sea level, marine erosional features appear above it and become increasingly abundant southwards. They comprise raised cliffs, platforms, caves, stacks and geos, most of which are a few metres above high-water mark (~1.5 m OD). Evidence that the features have been glaciated is not firmly established, except for one rock platform (~13 m) that passes beneath a cliff of till^{20,27-29}.

The Main Lateglacial Shoreline has not been studied northwards from Mull and the Oban area but it may be expected to pass below present sea level in southern Skye. Significantly, in northern Skye there are rock platform fragments at ~2.5 and

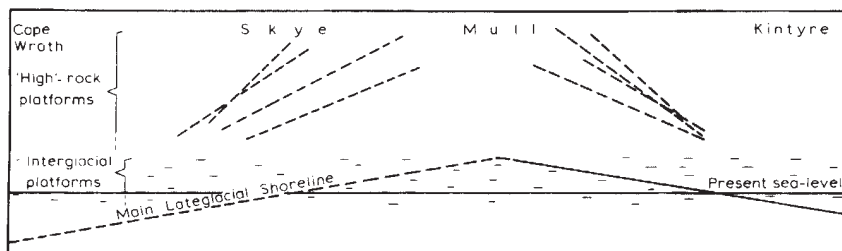


Fig. 2 Theoretical relationships of rock-cut shorelines along a north-south line in western Scotland.

~4 m while cemented beach deposits and the floors of cave entrances occur to altitudes several metres higher¹³.

These relationships suggest that interglacial marine erosion features occur not only at present sea level but also for some distance above it. As areas immediately below low-water mark have not been investigated, such features may also continue below present sea level. Thus I envisage a zone of interglacial marine erosion. Individual features may have been formed during more than one interglacial, just as the glaciated intertidal platform is being reshaped during the present interglacial. This view accords with the deep-sea record, which shows that eustatic sea level attained different altitudes during different interglacials.

Figure 2 shows theoretical relationships in western Scotland for the three sets of evidence considered above. No vertical or horizontal scale is given as available altitudinal data on rock platforms are inadequate, except for the southward slope above sea level of the Main Lateglacial Shoreline. The number and relative gradients of the proposed high-rock platforms are diagrammatic. The high platforms should slope down to present sea level (and below it): evidence for this is lacking, probably because the Inner Hebrides extend insufficiently far westwards, while mainland areas where this might happen (for example, North-west Scotland) have not been studied in detail. In these latter areas it may prove difficult to distinguish high-rock platforms from interglacial ones. The interglacial platform fragments are shown in Fig. 2 as unconnected short lines as they are at present not correlated with each other: as a group the fragments suggest the existence of horizontal or gently inclined interglacial shorelines.

At many points around the coasts of South-west England, Wales and Ireland one or more shorelines cut in bedrock have been identified from slightly below sea-level to ~15 m altitude. In the glaciated area the rock platforms often pass beneath till and in unglaciated terrain beneath head. It is accepted that the platforms are interglacial but opinions differ as to the interglacial(s) involved³⁰⁻³⁴. The occurrence of interglacial platform fragments within a limited range of altitude in many parts of the British Isles suggests that they are one set of features: it is therefore suggested that the interpretation proposed for the Scottish features applies elsewhere. An implication of this interpretation is that glacio-isostatic recovery has essentially ceased (unless glacial erosion has yet to be compensated).

In Scottish areas so far studied the rock platform and cliff associated with the Main Lateglacial Shoreline are clear and distinctive. They are clearly preserved beneath present sea level in at least part of eastern Scotland and hence are likely to be identifiable below sea level elsewhere. I have suggested³⁵ that the Main Lateglacial Shoreline may be represented by the sharp break of slope traced for 30 km at -35 to -40 m in Cardigan Bay³⁶ and by the pronounced erosional shoreline at -42 m identified off part of the coast of South-west England³⁷. The high rock platforms should relate to lower sea levels, perhaps of the order of -100 m.

If the interpretation of high rock platforms presented above is valid, then for much of the last glaciation (and, probably, other ones) the Scottish ice-sheet was far less extensive in western Scotland than previously envisaged. The ice that flowed east and south-east in the Outer Hebrides was at least 400 m thick at one point³⁸ and it has left pronounced glacial erosion forms⁷, implying that independent Outer Hebridean ice existed for a long time (or times). Many maps have been published showing an ice-sheet or ice-sheets terminating far to the south of Scotland (for example, at the latitude of south Wales or beyond). While such maps represent maximal conditions, they suggest that ice-sheets were normally extensive in the British Isles. The Hebridean evidence does not simply accord with this view for it suggests that, at least in Scotland, ice-sheets were normally of limited extent.

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Will the Sahelian drought end in 1985?

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The great variability of rainfall in the Sahelian areas of Africa is well documented for all time scales during the Quaternary and historical periods. Since the beginning of the twentieth century the variability can be quantified from many meteorological and hydrological surveys¹⁻⁶. The River Sénégal runoff gives a simplified view of the yearly mean rainfall over an extensive Sahelian and Sudanian area. The pattern of the annual river discharge over the nineteenth century shows short periods of drastic minimum followed by a slow return to longer periods of wet conditions with a double maximum. The mean time between the last three droughts is 31.3 yr with extreme conditions every 10.3 ± 4 yr. Extrapolating the curve of the mean annual modules suggests that the present drought should end in 1985 with full wet conditions being re-established in about 1992. If the same pattern continues, it is feared that a severe drought will occur around 2005.

In southern Sahara and northern Sahel the study of sediments, diatoms and pollens on cross-sections or cores of lacustrine deposits, has demonstrated that important environmental changes are frequent and repeated⁷⁻¹². This variability of the climate during the past 40,000 yr is one of the main features of the Quaternary in this part of Africa¹³. On another time scale, Nicholson¹⁴ and Maley¹⁵ have shown that there have been alternating wet and dry periods during the past 1,000 yr. The Lake Chad level reconstitution (Fig. 1) shows how the apparent period of fluctuations depends on the method of data collection. The resolution is related to the accuracy and length of time

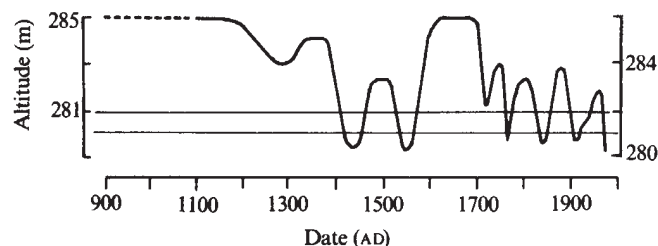


Fig. 1 Lake Chad level variations for the past 1,000 yr (from ref. 15). This curve synthesizes historical, geological and palynological data. Only the most important droughts have enough geological, historical or sociological impact to be recorded or memorized.

considered. On a 10^3 - and 10^4 -yr time scale, only important droughts have enough geological, historical or sociological impact to merit being recorded. This is why drought recurrence seems to be more frequent in recent times. Major famines and droughts during the past 300 yr, have been correlated by Nicholson¹⁴ who found they repeated at intervals of 72 ± 16 yr (56 – 86 yr between the maxima). For both the nineteenth and twentieth centuries it seems that one drought could occur during every generation. For example, a rainfall maximum was experienced around 1841 when the River Sénégal flooded into the Ferlo valley as far up as Linguère^{16,17}. A minimum rainfall appeared soon after, in 1848, when the Trarza from Mauritania crossed the River Sénégal to invade the Cayor and Oualo region¹⁸, at about this time the Lake Chad level was low¹⁵. The years 1870–1900 were relatively humid¹⁴. They could

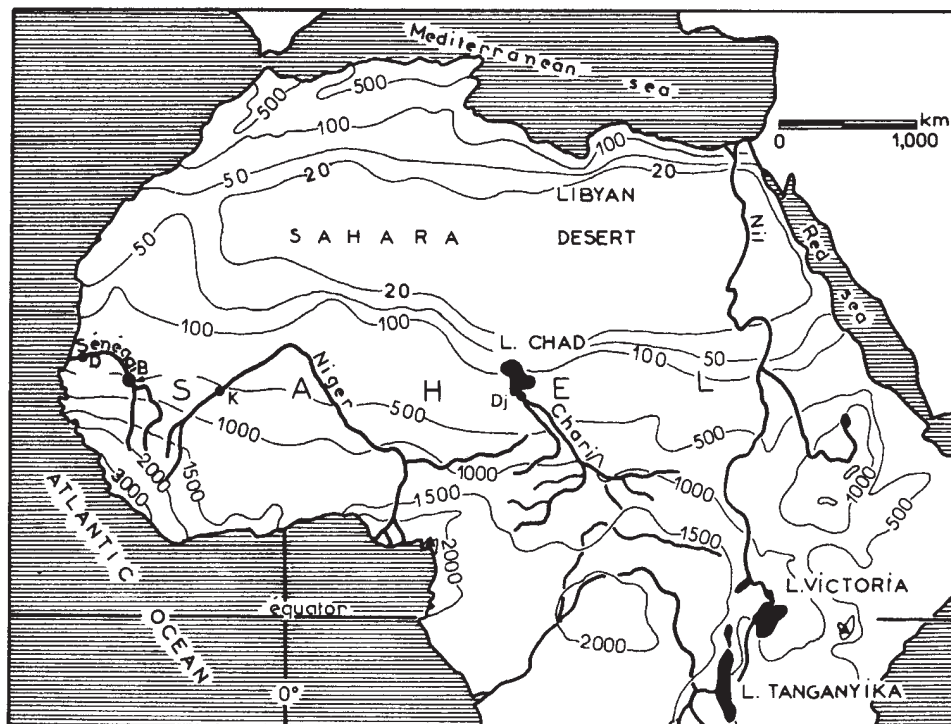


Fig. 2 Map showing the four main rivers flowing in Sahel. Isohyetes are from refs 28, 29. B, Bakel; D, Dagana; K, Koulikoro; Dj, Djamena.

Table 1 Mean annual runoff and maximum height of the River Sénégal at Bakel

Year	Runoff ($\text{m}^3 \text{s}^{-1}$)	H_{max} (m)	Year	Runoff ($\text{m}^3 \text{s}^{-1}$)	H_{max} (m)	Year	Runoff ($\text{m}^3 \text{s}^{-1}$)	H_{max} (m)	Year	Runoff ($\text{m}^3 \text{s}^{-1}$)	H_{max} (m)
1903	631	9.49	1923	755	11.05	1943	666	9.75	1963	666	10.11
1904	735	11.15	1924	1,244	12.20	1944	330	6.95	1964	970	12.56
1905	871	10.19	1925	839	11.00	1945	945	12.26	1965	1,048	12.48
1906	1,229	13.28	1926	520	8.00	1946	745	10.85	1966	841	11.70
1907	521	8.90	1927	1,074	12.25	1947	666	10.75	1967	1,037	11.94
1908	765	10.60	1928	903	11.70	1948	572	9.90	1968	397	8.94
1909	900	11.70	1929	898	11.70	1949	467	10.10	1969	764	10.11
1910	669	10.20	1930	837	11.00	1950	1,152	12.71	1970	542	9.70
1911	537	9.55	1931	738	10.70	1951	842	11.60	1971	598	10.72
1912	563	9.50	1932	768	11.20	1952	718	11.38	1972	263	6.24
1913	271	5.20	1933	831	11.70	1953	631	10.51	1973	361	8.38
1914	443	7.25	1934	699	11.60	1954	1,068	12.32	1974	760	11.91
1915	591	9.30	1934	1,164	12.35	1955	1,049	11.54	1975	602	10.14
1916	689	10.60	1936	1,234	12.70	1956	952	12.06	1976	470	6.96
1917	646	11.30	1937	644	9.90	1957	1,029	11.82	1977	324	7.06
1918	1,142	12.60	1938	807	11.80	1958	1,037	12.89	1978	523	7.87
1919	529	9.85	1939	559	9.65	1959	788	11.68	1979	301	6.20
1920	833	11.80	1940	430	8.75	1960	621	9.84	1980		8.66
1921	430	8.90	1941	417	8.95	1961	944	12.51	1981		
1922	1,218	13.19	1942	437	9.56	1962	769	10.80	1982		

Refs are given in Fig. 4 legend. The floodheight (H_{max}) is measured, after 1950, with an error of ± 1 cm. The runoff is then better than 5% accuracy. Before 1950 the error could possibly have a maximum value of 30 cm, the accuracy then fell from 5% for high values of runoff to 15% for low values (see ref. 4). From the measured maximum flood in Bakel, 8.66 m on 11 September 1980, it can be estimated that the hydrological year 1980 (ending in April 1981) will have a runoff of more than $500 \text{ m}^3 \text{s}^{-1}$, confirming the increase of 1976 and 1977.

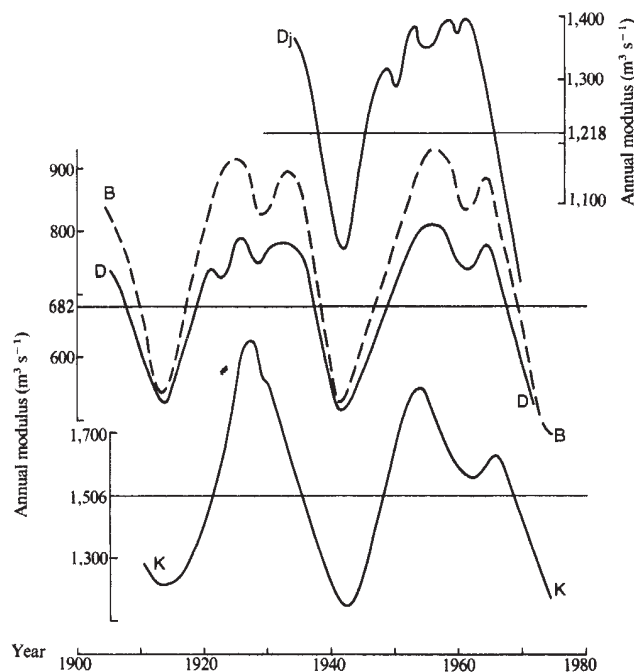


Fig. 3 Comparison of the pattern of river runoff in Sahel (7-yr running mean): Sénégat at Bakel (B) and Dagana (D), Niger at Koulikoro (K) and Chari at Djamena (Dj).

cover two periods of wet conditions because a break corresponding to a relative minimum is seen in Sénégat a short time before 1880 (ref. 14, Table 8.8).

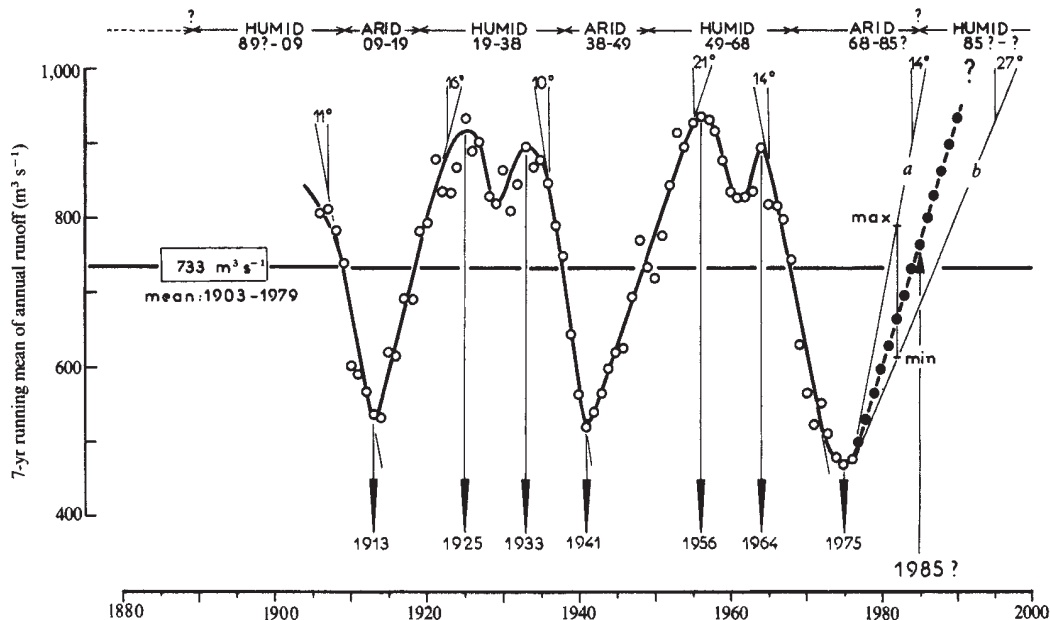
Since the beginning of the twentieth century regular meteorological and hydrological surveys^{2-6,19} have given a more quantitative and continuous set of data which illustrate the variability pattern of rainfall. The major rivers that flow through Sahel (Fig. 2), such as the Sénégat, the Niger, the Chari and partly the Nile, reflect the general annual and seasonal rainfall over a vast area of the Sahelian and Sudanian climatic zones. The annual discharges have been measured at several stations along the water course since the beginning of this century. The variation of the annual discharge shows a similar pattern for the various rivers² (Fig. 3): three drastic droughts clearly separated by two longer periods of humid conditions showing fluctuations

mainly induced by flood plains evaporation, shape, lithology, soil, and so on. The River Sénégat has been taken as a typical example, and the Bakel hydrological station chosen because it is upstream from the main flood plains which experience great evaporation. The Bakel station (Table 1) gives a general idea of the mean rainfall over Sahelian and Sudanian zones. In both zones rainfall is a consequence of the North-South shifting of the monsoon. Meteorological data show that the annual rainfall in Sahel and Sudan fluctuates in the same direction^{20,21}.

To attenuate the effect of short-term variations, apparently 2 and 2.5 yr periods, curves have been constructed with various running means. We use the 7-yr running mean (Fig. 4) because the high and low points of this curve show the best coincidence with the years of maximum and minimum aridity. But similar results would be obtained with a 5-yr running mean more frequently used by hydrologists^{5,19}. The curve (Fig. 4) shows several repeated characteristics. The slopes of the subvertical branches indicate that the onset of a drought is more rapid than the return to a wetter condition. Furthermore, each wet stage has a double maximum. This means that favourable conditions last ~18 yr, that is twice as long as arid periods characterized by peaks of ~8–12 yr. The drought maximum seems to recur after 31 ± 3 yr. If we consider only major events differing from the mean by more than 20%, the past 76 yr have seen seven of these extreme (maxima or minima) situations. They are separated by 8–15 yr; mean recurrence time is 10.3 yr. During the same period the solar cycle is 10.4 yr. Although the effect of solar cycles on climate is very controversial²², indirect and complex relations could result from high atmosphere ionization^{23,24} or geomagnetic field influence from solar wind^{25,26}.

The period of measured data (76 yr) is too short to establish a firm cyclicity in the drought appearance. Neither the nineteenth century observations, nor the records of the past 300 yr deny that a more or less dramatic drought could return at every generation, as claimed by the salt and trona producers whose livelihood depends on water table fluctuations²⁷. If such a pattern continues some characteristics of the curve (Fig. 4) can be used to predict a date for the next rainfall maximum. The slope of the discharge curve during the transition to wet conditions can be used to estimate a slope between 14 and 27° for the next transition (Fig. 4). Consequently the long term average runoff should be reached at ~1985 (± 2 yr) and the return to a maximum wetness at around 1992 (± 4 yr). However, this favourable period will undoubtedly end and a new drought will occur towards the beginning of the next millenary; at ~2005.

Fig. 4 Mean annual modulus of River Sénégat runoff measured at Bakel (7-yr running mean). The annual discharges have been compiled from documents of Senegal-Consult (1903–68), ORSTOM (1903–64), M. Juton (1969–71), A. Hamdinou (1971–75), ORSTOM (1975–78), Senegal Hydrological Service (1978–80). For predictive part of the curve (1977–92) *a* has the maximum slope (14°) of the preceding humid/arid change (the return to humid is slower than the humid/arid transition); the slope of *b* is given by deviation from the slopes of the two preceding fluctuations. The projected points are the mean annual modulus



$$D_{(year x)} = \frac{R_{(x-3)} + R_{(x-2)} + R_{(x-1)} + R_{(x)} + R_{(x+1)} + R_{(x+2)} + R_{(x+3)}}{7} \text{ where } R = \text{runoff. The ordinate indicates the mean annual modulus } D.$$

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Geological evidence against major displacement in the Nares Strait

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Early continental drift^{1–3} and later plate tectonic reconstructions^{4–14} indicate left lateral (sinistral) movement along Nares Strait of between 150 and 400 km during the Cretaceous–Tertiary. A basic concept in such reconstructions is the substantial part played by seafloor spreading in the origin of Labrador Sea–Baffin Bay. Until recently, geological knowledge of the area adjacent to Nares Strait was insufficient to put tight constraints on displacement along Nares Strait (Fig. 1). Of seven independent lines of evidence from bedrock geology, termed markers, all are consistent with sinistral net displacement of Greenland along Nares Strait of 0–25 km. The three most imprecise markers can be reconciled with maximum movement of 100 km.

This refutes conventional plate tectonic reconstructions which suggest movement of 150 km or more. New mechanisms should now be considered to explain the broad area of oceanic crust in Baffin Bay.

The foundation of the continental crust is the Precambrian shield that crops out in much of the southern part of the Nares Strait region (Fig. 1)^{15–19}. In both Ellesmere Island and Greenland the shield consists of a high grade gneissic and metasedimentary terrain; a distinctive suite of acidic to basic igneous rocks has locally been transformed by Hudsonian deformation into complexly folded orthogneisses. Both regions contain marble-rich metasedimentary rocks; in Greenland these are mapped in the north as continuous linear, steeply inclined tracts up to 2 km thick that strike south-west near the coast of Nares Strait (Fig. 2). Similar marbles occur throughout the nunatak terrain of southeastern Ellesmere Island and these also form a metasedimentary-rich zone.

The marble-rich tracts in Ellesmere Island and Greenland (marker 1) are inferred to be part of the same supracrustal belt. No similar belt is known in the crystalline terrain elsewhere in the region of eastern Devon Island and northern Baffin Island in Canada or in the Thule and Melville Bugt areas in Greenland. This lithological continuity is evidence for correlating the shield areas of Ellesmere Island and adjacent Greenland directly across Nares Strait. No displacement thus need be invoked, and

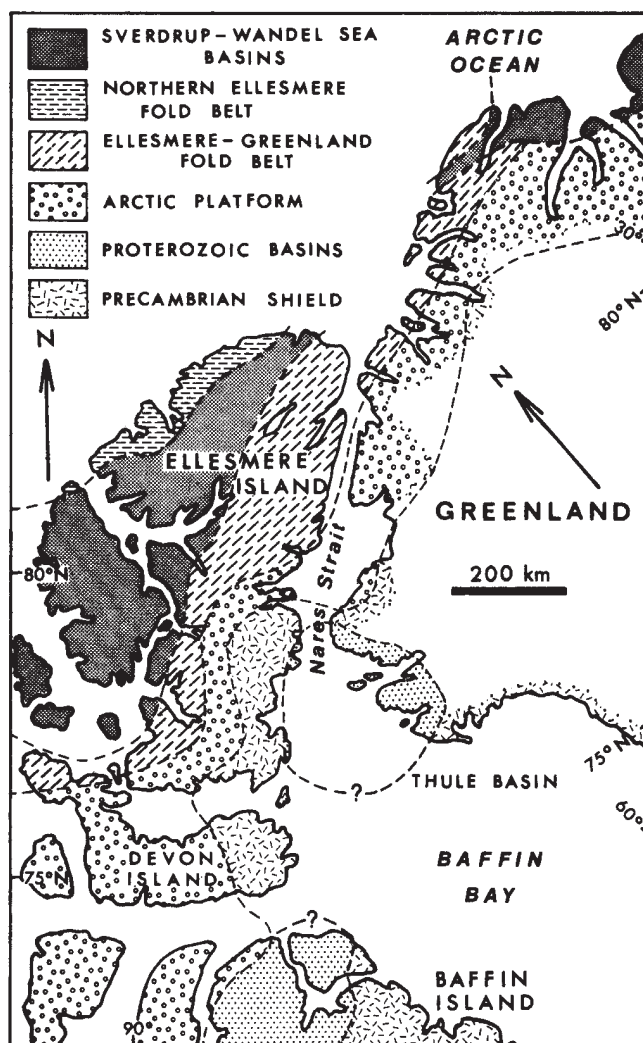


Fig. 1 The setting and main geographical features of the Nares Strait region together with the main geological provinces.

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although this is an imprecise marker, sinistral displacement in excess of 100 km is considered unlikely.

The Thule Basin (marker 2) is an intracratonic basin spanning southernmost Nares Strait (Fig. 2) and consists of several formations of Proterozoic sedimentary and volcanic rocks, making up the Thule Group in north-west Greenland¹⁸⁻²¹. Some of the same formations occur on Ellesmere Island¹⁵⁻¹⁷. The Thule Group is unmetamorphosed and overlies the Precambrian Shield with profound unconformity. In Greenland the Thule Group is several kilometres thick; it consists essentially of a lower sequence of clastic sediments with basalt flows and sills, and an upper sequence of sandstones, shales and carbonate rocks. Only the lower sequence is present on Ellesmere Island¹⁷. Unit to unit matching, with excellent marker beds, allows a reliable correlation between Ellesmere Island and Greenland^{17,21}. The strata thin northwards onto the Bache Peninsula Arch both in Greenland and Ellesmere Island, defining the northern margin of the Thule Basin. No apparent displacement of this margin occurs across Nares Strait²²⁻²⁴, but sinistral movements up to ~50 km could be accommodated.

The Bache Peninsula Arch²⁵ (marker 3) is a structural feature composed of Precambrian Shield rocks that must have been relatively high structurally, at least as early as late Proterozoic time. Thule Group strata over the arch in both Ellesmere Island and Greenland have a remarkable similarity in thickness and lithology, as illustrated by the classic platform sequences on Bache Peninsula and in Inglefield Land²⁶⁻²⁸. The Bache Peninsula Arch is also reflected in thicknesses of Cambrian to Ordovician rocks in Ellesmere Island and Greenland. The arch is a broad feature, not narrowly delineated on either side of Nares Strait, but its present trend across the strait is consistent with its having had the same trend in at least Proterozoic and early Palaeozoic time. However, as it is an imprecise marker some displacement could be accommodated, though it cannot have been more than 100 km in a sinistral sense.

The Franklinian Geosyncline is a linear basin that extends northeastwards through the Canadian Arctic Islands and northern Greenland^{18,29-31}. The geosyncline contains a thick column of Upper Proterozoic to Upper Devonian sediments. Several linear features of the Franklinian Geosyncline (markers 4-6) are pertinent to displacement along Nares Strait.

The Franklinian Geosyncline passes southeastwards into equivalent rocks of the Arctic Platform. The boundary separating them is a hinge-line, across which sedimentary thicknesses increase rapidly northwards. The hinge-line migrated laterally from time to time on Ellesmere Island³¹ and for the most part it coincides with the south-east boundary of late Palaeozoic folding (marker 4, Fig. 2). This marker is a structural boundary which might have an enhanced value because it generally coincides with the hinge-line that existed before the strait formed.

The south-east limit of folding (marker 4) is well delineated both on Ellesmere Island^{29,31} and in Greenland^{18,24}. The limit of folding has an apparent offset along Nares Strait and this has been used^{32,33} to suggest that there was at least 250 km of left lateral strike-slip displacement. It was suggested^{32,33} that the southernmost folds of Ellesmere Island originally continued directly across the strait to the southernmost folds in Greenland, and that the present apparent offset was created by left lateral strike-slip faulting after the folding. However, this is not substantiated by the detailed structural geology which shows that in eastern Ellesmere Island, close to Nares Strait, fold trends swing markedly from easterly to northeasterly^{29-31,34-36}. Trends of individual folds run parallel to Nares Strait for some distance. Similar swings in fold trends occur in northern Greenland^{18,24}, and farther west in Ellesmere Island²⁹⁻³¹. The swings may coincide with salients and re-entrants in the hinge-line at the margin of the original sedimentary basin. We believe that the south-east limit of folding reflected the change in fold trends in easternmost Ellesmere Island, and had an original bend from Ellesmere Island northwards along the line of Nares Strait itself (Fig. 2), and then bent again eastwards into Greenland. Marker

4 exhibits its closest match across Nares Strait with models of displacement between 0 and 50 km, while displacements above 50 km create unacceptable divergences in fold trends.

Two important Lower Palaeozoic facies boundaries occur on Ellesmere Island and in Greenland. Marker 5 represents the Cambrian to Upper Ordovician trough margin, and marker 6 the Lower Silurian to Devonian facies front between platform carbonates and mudstones. The Cambro-Ordovician facies boundary on Ellesmere Island (Fig. 2) separates thick, shallow water carbonates of the miogeocline to the south-west from

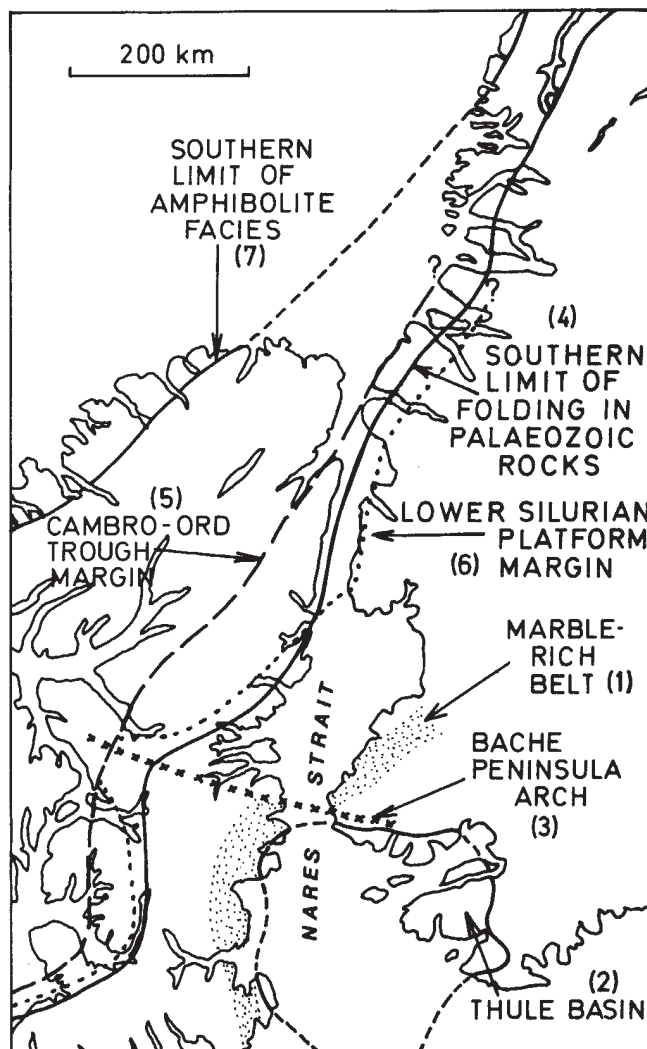


Fig. 2 Geological 'markers' that occur on both sides of Nares Strait that are useful for assessing displacement.

equivalent mudstones, resedimented conglomerates, cherts and turbidites of the Hazen Trough farther north-west³⁷. This marker is linear and a similar feature in eastern North Greenland is the site of faults^{38,39}. Facies relations on northern Hall Land and Nyeboe Land together with the linear nature of the coastline suggest that this is the site of marker 5 in western North Greenland. The linear boundary in Ellesmere Island is on direct strike with the similar boundary in northern Greenland, and suggests that only small displacements can have taken place; displacement in excess of 50 km can probably be excluded.

The Lower Silurian platform margin (marker 6) represents the facies front between the clastics of the Hazen Trough and the carbonates of the Franklinian Miogeocline and has a position to

the south-east of marker 5. The location of this marker is shown in Fig. 2 in Greenland⁴⁰⁻⁴² and Ellesmere Island⁴³ during Middle Llandovery time. A feature of the position of this boundary with time is its southerly migration. The diachronous southerly spread of the mudstone facies fringing the Hazen Trough started during the latest Ordovician in Ellesmere Island and reached western North Greenland in the Early Silurian^{41,42} indicating facies continuity across Nares Strait. The facies boundary as depicted in Fig. 2 lasted until Late Silurian time in Greenland and until Devonian time on Ellesmere Island. This is perhaps the best of all the markers in that it is a precisely dated, discrete, vertical boundary and crosses Nares Strait at one of its narrowest points. It strikes across the strait without offset, but because of the meandering nature of facies fronts sinistral displacement of up to 25 km cannot be ruled out.

Fold and metamorphic intensity increases northwards in Ellesmere Island and northern Greenland. The Pearya Geanticline, a linear tectonic feature cored by metamorphic and igneous rocks, trends northeastwards on northern Ellesmere Island. The events in the long tectonic history of the Pearya Geanticline are well established³⁰. A region of high-grade amphibolite facies metamorphism (Fig. 2) occurs in the geanticline on northern Ellesmere Island^{44,45}, and the southeastern margin of amphibolite facies rocks is given here as marker 7. Amphibolite facies rocks also occur in northern Greenland in a narrow, intensely deformed zone in the extreme north⁴⁶ where their southeastern margin is regarded as marker 7. The amphibolite-grade metamorphic belts of northern Ellesmere Island and North Greenland are fundamental, deep rooted features, a type which is not prone to rapid changes in trend. If the original boundary was approximately straight, which seems reasonable, the present situation of the amphibolite facies rocks (Fig. 2) is consistent with minor displacement along Nares Strait. However, the two regions are separated by a wide expanse of water and displacements of up to 100 km could be accommodated with approximately linear connections between the two areas; models with greater displacement become increasingly tenuous.

Each of the seven markers has some bearing on the amount of maximum net displacement that can have occurred along Nares Strait, the amount varying with the nature and the reliability of the geological data defining the marker. Three imprecise markers could accommodate up to 100 km displacement, while three other markers could be reconciled with up to a maximum of 50 km sinistral movement. All the markers are consistent with net sinistral displacement in the range 0–25 km; marker 6, which permits the narrowest range of possible movement, is also considered to be the most precise of the markers. We therefore conclude that net strike-slip displacement along Nares Strait was minor, and not greater than 25 km.

Some of the above markers have been used^{32,33} to support displacements of up to 300 km along Nares Strait. The correlation of the south-west limit of folding (marker 4) directly across the strait has been mentioned above. The Thule Basin deposits of Greenland (marker 3) have been correlated in such models with Proterozoic deposits of northern Baffin Island (Fig. 1). There is a general similarity between these strata, and also strata in other Proterozoic basins on the northern margin of the North American craton; however, models with such large displacements violate the detailed correlation of the Thule Basin rocks in Greenland and Ellesmere Island. Correlation of faults and thrusts on the two sides of Nares Strait have also been suggested in models with large displacements, but some of these structures are associated with important Tertiary movements^{25,31} and are thus of little value in determining the position of land masses in pre-drift reconstructions. Faults and thrusts in Greenland can be correlated with other, on-line, dislocations in Ellesmere Island without invoking displacement.

The linearity and length of Nares Strait does, however, suggest that it is the site of a movement zone. Indeed, we believe that motion probably has occurred along it, particularly in view of the fault zone of Judge Daly Promontory^{25,47-49} and the

suggested origin of the ocean basins surrounding the region. Left lateral strike-slip movement of 19 km on the fault zone is estimated by Mayr⁴⁹ from offset of limbs of an intersected fold.

The Nares Strait is an important element in plate tectonic reconstructions of the Northern Hemisphere. Geological data placing constraints on the amount of net movement that may have occurred along the strait have wide geological implications⁵⁰. Thus, without invoking a complicated movement history for Nares Strait (one might speculate dextral displacement(s) in the late Palaeozoic or/and Mesozoic time followed by sinistral displacement(s) associated with the formation of the surrounding oceans) the geological evidence conflicts with the widely accepted origin of Baffin Bay^{8,12,32,51-53}, in which lateral movement by seafloor spreading approximates the distance separating the present continental shelves. Rigid-plate models have failed to produce an all embracing solution for the origin of Baffin Bay (and also Labrador Sea) that takes into account the geological restraints of the lands bordering the oceans. We would like to see a closer coordination of the onshore geological data into the discussion of the origin of the surrounding oceans that are mainly based on offshore geophysical data.

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Molecular detail in electron micrographs of quaterrylene $C_{40}H_{20}$

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The electron microscopy of organic compounds is beset by the problem of radiation damage due to the electron beam¹. This radiation sensitivity is somewhat reduced in aromatic systems² and organometallic complexes³⁻⁵ but it, nevertheless, limits the fineness of detail which can be resolved in images from these types of materials. Previous studies of the more stable compounds have concentrated on those where molecular superimposition normally occurred on a major crystallographic axis. The compound quaterrylene, $C_{40}H_{20}$, does not have such a projection axis and is therefore more typical of most organic crystals. We report here results from the examination of quaterrylene⁶ in the Cambridge University 600-kV high resolution electron microscope (HREM)^{7,8}. These results provide direct information about intermolecular stacking and, after lattice averaging, show detail at the level of the six-membered aromatic rings within molecules of this material.

The limitations posed by radiation damage in the electron microscope can be partially circumvented by spatial averaging over many unit cells, the use of sensitive photographic emulsions such as X-ray film, and recording images from previously unexposed areas^{1,2,9,10} (minimum exposure technique¹¹). Clearly, spatial averaging does not provide direct information about nonperiodic features, although it permits structure determination from sample volumes many orders of magnitude less than other techniques such as X-ray diffraction and it was of particular value to the present study where the specimen consisted of very small domains (~ 10 nm)³. Furthermore, X-ray film has a superior modulation transfer function to that of normal electron microscope film^{12,13} which is of particular relevance in

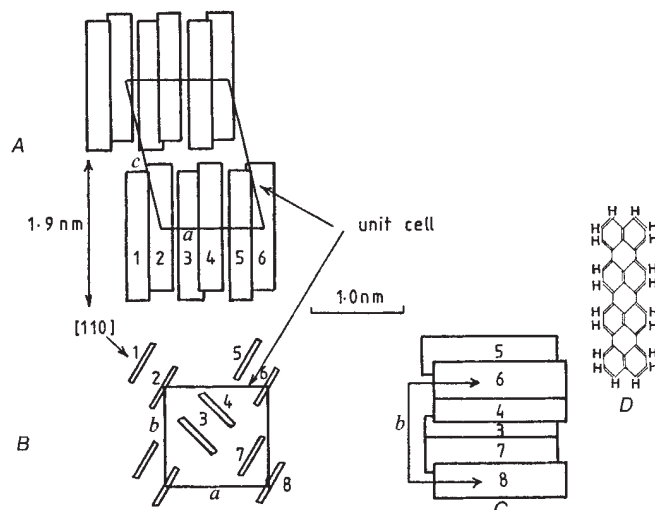


Fig. 1 Crystal and molecular structure of quaterrylene. Molecules are represented as rectangles in crystal projections, numbering referring to the same molecule in each projection. A, *b*-axis projection, two rows of molecules; B, end-on view of molecules in orthogonal *c*-axis projection; C, *a*-axis projection; D, molecular structure.

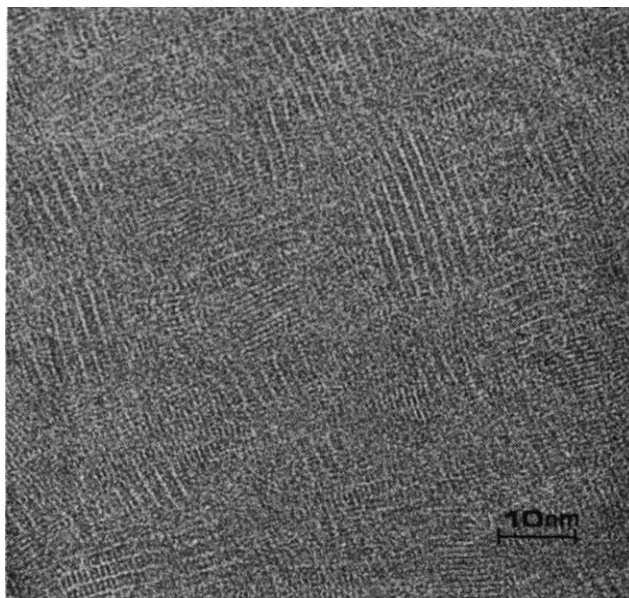


Fig. 2 Low-magnification micrograph of a crystalline mosaic of quaterrylene evaporated onto KCl (100).

studies of beam-sensitive specimens due to the very small electron exposures required to reach optimum optical density. Higher accelerating voltages have also been reported to reduce the rate of damage in some materials¹⁴. Although examination of this effect was not the purpose of the present work, the improved contrast transfer function (interpretable resolution) of the microscope used, relative to conventional 100-kV machines¹⁵, should lead to an enhanced signal-to-noise ratio and hence improved object definition.

Quaterrylene is a planar polynuclear aromatic hydrocarbon whose molecular structure is shown in Fig. 1D. The crystal structure¹⁶ is monoclinic with $a = 1.115$, $b = 1.063$, $c = 1.924$ nm, $\beta = 100.46^\circ$, $z = 4$ and space group $P2_1/a$. Projections of this structure are shown in Fig. 1A, B, C with the molecules represented by rectangles. These projections show that there was not exact molecular superimposition on any major axis, but that a {110} projection—despite its consisting of molecular pairs at different inclinations—may give rise to near superimposition of regions of high electron density (such as the naphthalene moieties) that would provide information about molecular positions and orientation.

Vacuum evaporation of quaterrylene onto a KCl (001) substrate provided a crystalline mosaic of <10 nm thickness (Fig. 2) with small crystals oriented around the (110) plane. This plane aligns approximately parallel to the substrate. Deviations from this orientation were within a few degrees and occurred randomly. The *c*-axis projection of the crystal structure (Fig. 1b) shows that images should be obtained of the molecular columns down a {110} axis, with molecules having their molecular planes slightly inclined, at 9° and 81° , to the direction of the incident electron beam.

Operation of the HREM for these studies has primarily been at an accelerating voltage of 500 kV, with magnifications typically of around $\times 100,000$. Minimum exposure techniques were used, with a low-inertia beam shutter above the specimen¹⁷ blanking off the beam while a fresh specimen area was shifted into place ready for photographic recording. The optimum defocus (~ -390 nm) was difficult to estimate experimentally because of the relatively low magnification, the low specimen contrast and the low beam intensity. Furthermore, there were often slight variations in vertical position across the specimen. Consequently, three exposures with focal differences of 75 nm were normally taken of each area of the specimen. Typical electron doses for an exposure were in the range 1,000–2,000 electrons nm^{-2} . This dose causes severe damage in aliphatic

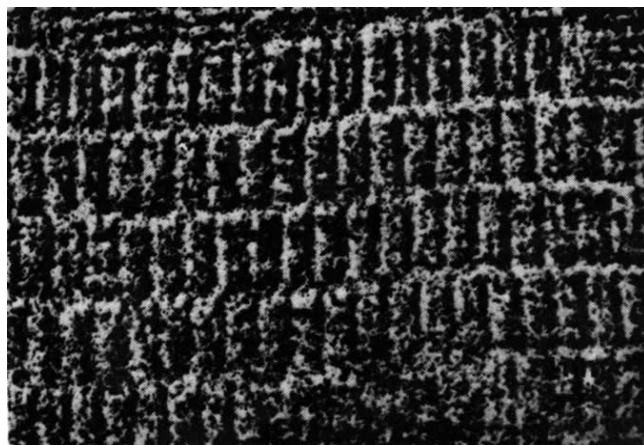


Fig. 3 Image of four rows of molecules in (110) projection. Slight variations in inclination down (110) giving rise to differences in contrast along each row and the curve in the 1.9-nm lattice spacing probably arising from the fusion of two crystals nucleated at different points on the KCl substrate.

systems¹⁸ but for an aromatic hydrocarbon should not excessively limit the image resolution. Considerable blackening of the reverse side emulsion occurred due to penetration of the high-energy electron beam through the film backing, as well as back-scattered radiation from the film holder. As this low resolution information obscured the primary image, it was normally removed before serious analysis of image features was undertaken.

As a consequence of the very low electron dose used for recording, as well as the minimal exposure of the sample to the electron beam, our images revealed details of quaterylene molecules at a level hitherto unattained in previous images of aromatic hydrocarbons in the electron microscope; optical diffractogram analysis of the micrographs indicated the transfer of specimen information to resolutions better than 0.35 nm. Figure 3 shows the projection of the molecular columns down a {110} axis whilst Fig. 4 shows structure that dimensionally corresponds to the molecular projection as drawn. (The signal-to-noise ratio of the latter image has been enhanced by a factor of ~3 by photographic superimposition 10 times.) The pattern of contrast observed can be related to the positions of the double and single rings of quaterylene occurring within the (110) column, although the crystal structure of this material, as pointed out above, precludes the exact superimposition of molecules down this axis.

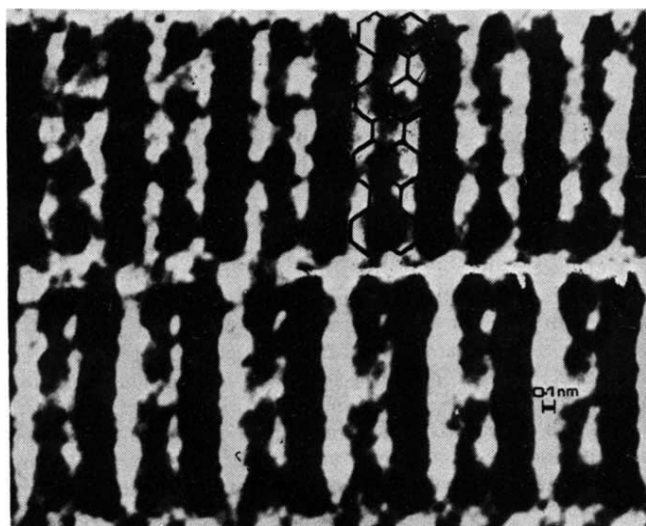


Fig. 4 Image obtained after averaging micrograph at close to (110) projection, with molecular structure superimposed.

We cannot yet judge whether the improved molecular detail in our micrographs is a direct consequence of the increased beam voltage, as no comparable study of quaterylene has been undertaken at lower voltages but we hope soon to observe anthanthrene for which such information exists². Certainly, quaterylene is slightly more beam stable than many hydrocarbons, and is comparable with copper phthalocyanine and nucleic acid bases. For more sensitive materials some sort of low temperature specimen stage, as well as the other methods used here, may be necessary to resolve detail on the molecular scale^{19,20}.

The ability to resolve detail in images of aromatic hydrocarbons to better than 0.35 nm, would permit molecules of similar size to quaterylene to be located and their relative configurations to be determined at grain boundaries and other nonperiodic regions of thin crystals—of particular relevance is the van der Waals distances of ~0.35 nm between molecules in these materials where no other intermolecular forces are present. An examination of thin film annealing, grain boundary structure and dislocations in quaterylene has certainly proved possible—a full report will be published elsewhere. Such direct information about morphological features should prove essential to any complete explanation for a wide range of physical properties (such as the electrical conductivity in thin quaterylene films) of these materials.

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Model protocells photochemically reduce carbonate to organic carbon

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Synthetic cell-sized organic microstructures effect the long-wavelength UV photosynthesis of organic products from carbonate. Formaldehyde is the most abundant photoproduct and water is the major proton donor for this reduced form of carbon. The apparent quantum yield is ~10⁻⁵ carbon atoms per incident UV 254-nm photon. We show here that these results for model phase-bounded systems are consistent with the postulate that metabolism of progenitors to the earliest living cells could have been, at least in part, photosynthetic.

A 'protocell' is here defined as an organic cell-sized phase-bounded structure which arose from organic precursors of abiological origin. We propose that phase-bounded structures of organic composition were probably progenitors to the first living cell, and that such protocells were in existence for perhaps 500–1,000 Myr (refs 1, 2).

Protocells have been the subject of numerous modelling studies^{3–8} which have raised several questions, such as the plausibility of protocell formation in primordial conditions and the suitability of protocells to evolve towards biological forms. However, all protocell models mirror features of contemporary cells such as size and the presence of phase boundaries.

The existence of phase boundaries necessitates that there is a difference in chemical composition and energy level between protocell interior and the environment. Viewing metabolism as changes in concentrations and reaction rates of compounds across phase boundaries, then the beginnings of cellular metabolism coincide with the formation of protocells. Many alternative models for protocell metabolism can be added within these general constraints. We have designed a model system which demonstrates the photosynthesis of formaldehyde and other organic products by the long-wavelength UV irradiation of aqueous suspensions of organic model protocells and carbonate under a nitrogen/hydrogen atmosphere.

Phase-bounded organic microstructures were prepared from quenched spark discharges through gas mixtures of N₂ (50 mm), CH₄ (200 mm) and CO₂ (50 mm) on a distilled water surface as described elsewhere^{1,8}. The structures' form can be disrupted by freeze-thawing. The osmotically sensitive nature of these microstructures is revealed by microscopic examination: salt equilibrated (1 M NaCl) suspensions which are shocked by sudden exposure to distilled water will swell and burst. Up to 60% of the total recovered organic carbon is phase-bounded. These microstructures display complex morphologies and occur in a range of sizes 1–20 µm in diameter. Their number increases autocatalytically during synthesis, which indicates that self-assembly of new structures on surfaces of pre-existing structures occurs¹.

Microstructures were washed by centrifugation in distilled water and stored frozen or used immediately. For all carbonate reduction experiments, septum-stoppered quartz tubes 12 × 100 mm were used containing 2 ml of microstructure suspension (about 7 mg dry wt microstructures) and a 6-ml headspace which was filled, after repeated flushing, with 710 mm N₂ and 50 mm H₂. A small Teflon-coated magnetic flea was used to stir the mixture continuously. ¹⁴C-sodium bicarbonate at a specific activity of 50 Ci M⁻¹ (Research Products International) was added and reaction mixtures were irradiated in a Rayonet photochemical reactor which contained 16 low-pressure mercury 8-W lamps with Vycor envelopes. UV 254-nm intensities were measured by thymine dimer actinometry using the same size quartz tubes⁹ and were 249 erg s⁻¹ mm⁻². The UV 254-nm radiation incident on exposed surfaces of the quartz reaction vessels was 49.9 × 10³ erg s⁻¹. Temperature at the reaction vessel site was 27 °C. Unirradiated control experiments were wrapped in two layers of aluminum foil and placed in the photochemical reactor.

After 24 h exposure, the reaction vessel was evacuated, then acidified with 1.5 ml 11 M HCl, flushed with N₂ and degassed under vacuum for 30 min to remove unreacted carbonate. The sample was centrifuged at 12,000g for 5 min to yield a soluble and a particulate phase: the latter was rinsed several times and washings were combined with the soluble phase. Both particulate and soluble fractions were assayed for ¹⁴C activity by liquid scintillation counting. The sum of these two fractions' activities is termed the total organic carbon produced in the reaction from labelled carbonate.

Generally 30% or less of the total recovered organic carbon was found in the particulate fraction: the balance was soluble organic carbon. These results are presented in Table 1. The data show that carbonate is photoreduced to organic carbon. The reaction requires the presence of organic microstructures and UV 254-nm radiation (see control experiments 1 and 2). Higher yields of reduced carbon are obtained if the microstructures are intact and not disrupted by freeze-thaw cycles (experiment 4). The apparent quantum yield (the number of carbon atoms reduced to organic carbon per incident UV 254 nm photon) is as high as 2.4 × 10⁻⁵. The actual quantum yield of this reaction is higher, as no corrections have been made for photons actually adsorbed by the reaction mixtures.

The soluble organic fraction was concentrated by partial lyophilization and separated by thin-layer chromatography (TLC) on silica gel-H in various solvent systems. Bands 1 cm wide were scraped from the plates and ¹⁴C activity measured by liquid scintillation counting. About 70% of the applied label was found to migrate with *R_f* values identical to those of an authentic ¹⁴C-formaldehyde standard in the following solvent systems: (1) methanol/water-saturated (NH₄)₂CO₃, 3/1; (2) 2-butanol/water/formic acid, 17/2/1; (3) chloroform/methanol/4.4 M NH₄OH, 2/2/1, (4) CH₂Cl₂/methanol, 1/1; (5) acetone/ethyl acetate, 9/1. Similarly, a glyoxal standard is coincident with a less abundant ¹⁴C-labelled product in the same five solvent systems. Except for some amino acids the balance of the labelled material has not been positively identified.

Total soluble organic products from several experiments were concentrated and fractionated by chromatography on 8 × 150 mm AG 50 × 8 H⁺ ion exchange columns. The water rinse (four-bed volumes) released 90% of the input counts as the neutral and basic fractions. A final rinse of four bed volumes of 1 M NH₄OH released 9% of the input counts (total released 17,620 d.p.m. ¹⁴C), the acidic fraction; while 1% of the input label remained bound to the column. The acidic fraction was concentrated and separated by two-dimensional silica gel-H TLC in (I) methanol/chloroform/17% NH₄OH, 2/2/1, and then (II) *n*-butanol/acetic acid/water, 8/2/2. The plate was stained with ninhydrin to detect visually amino acid spots. Then the plate was divided into 22 equal area zones, each of which was eluted and measured for ¹⁴C activity. Seven well-defined ninhydrin-positive spots were observed with ¹⁴C activities of 2,600, 1,200, 660, 510, 460, 200 d.p.m. of the total 17,620 d.p.m. placed on the plate. The balance of the labelled ninhydrin-negative material migrated with an *R_f* > 0.9 in both solvent systems. The most intense ninhydrin positive spots which bore ¹⁴C label corresponded to: glycine (2,600 d.p.m.), alanine

Table 1 UV photochemical reduction of carbonate by model protocells

Experiment no.	Total input ¹⁴ C label (mCi)	Total recovered ¹⁴ C organic (mCi × 10 ⁻⁶)	Organic C in product (mM × 10 ⁻⁹)	No. C atoms reduced (erg ⁻¹ cm ⁻² × 10 ³)	Φ _{app} : apparent quantum yield
1	0.5	0.36	7.2	12	0.09 × 10 ⁻⁶
2	0.5	0.045	0.9	—	—
3	0.4	92.66	1,853.2	3,096	24.2 × 10 ⁻⁶
4	0.125	3.36	67.2	112	0.88 × 10 ⁻⁶
5	0.5	20.35	407.0	680	5.32 × 10 ⁻⁶

Experimental procedures are detailed in text. Experiment 1, no organic microstructures added; experiment 2, no UV irradiation; experiment 3, all reactants present (microstructures in distilled water, ¹⁴CO₃²⁻, N₂/H₂ head space); experiment 4, all reactants present but microstructures disrupted by repeated freeze-thaw cycles; experiment 5, all reactants present but pH 8.1 50 mM phosphate buffer replaced distilled water. The freeze-thaw cycling of structures before irradiation disrupted mechanically the phase boundaries of these model protocells.

(1,200 d.p.m.), β -alanine (1,100 d.p.m.) and valine (660 d.p.m.). Thus ~3.3% of the total ^{14}C -labelled soluble products appear to be amino acids. About four other ninhydrin-negative ^{14}C -labelled spots (450, 2,500, 1,850, 1,500 d.p.m.) remain unidentified.

The source of nitrogen for these amino acids is undetermined and could be derived either from microstructure organic material or from atmospheric N_2 .

Freshly made organic microstructures washed twice in H_2O were pressed dry and 9 mg were placed in a quartz reaction vessel with 1 mg of unlabelled NaHCO_3 . The vessel was flushed with N_2 and filled with 710 mm N_2 , 50 mm H_2 and 100 μl $^3\text{H}_2\text{O}$ (100 mCi at a specific activity of 1 Ci ml^{-1} , Research Products). The mixture was stirred and irradiated for 24 h as in previous experiments. After irradiation, the reaction mixture was lyophilized and resuspended in H_2O for three cycles to remove unreacted tritium label. Then 2 ml of H_2O containing 56,000 d.p.m. ^{14}C -labelled formaldehyde (specific activity 30 Ci M^{-1}) were added as an internal standard. $\text{Ba}(\text{OH})_2$ in excess was added to precipitate carbonate, the supernatant was lyophilized to 50 μl and applied to two silica gel-H plates for development in CCl_2H_2 /methanol solvent systems at 95/1 and 1/1 (v/v). Zones 2 cm wide were eluted and measured for both ^{14}C and ^3H activity by liquid scintillation counting. Total organic ^3H recovered from the reaction was 1.113×10^6 d.p.m. Of this, >70% was found to co-elute in both TLC solvent systems with the internal standard formaldehyde. The tritium label experiment shows that 1.67×10^{-5} mM ^3H was found in formaldehyde, while a typical carbon label experiment (as experiment 3, Table 1) yields 1.85×10^{-6} mM carbon in formaldehyde. As the experimental recovery of formaldehyde is low and variable, these two experiments indicate only qualitatively that many formaldehyde protons appear to be derived from water.

Routine microbiological surveys for contamination, conducted by placing aliquots of organic microstructure suspensions directly onto agar media (trypticase soy agar, nutrient agar, yeast extract agar) were negative, as were quartz tube reaction mixtures. Unirradiated reaction mixtures containing ^{14}C -labelled NaHCO_3 yielded only traces of presumptive labelled organic products (Table 1, experiment 2, 100 d.p.m. recovered). UV-irradiated reaction mixtures which contained ^{14}C label but no microstructures yielded some presumptive organic material (Table 1, experiment (1), 800 d.p.m. recovered), but this value is orders of magnitude less than in experiments which include microstructures.

Hong, Hong and Becker¹⁰ had previously shown that amino acids and other biomolecules can be formed from single carbon precursors by photochemically generated 'hot' hydrogen atoms. Sagan and Khare¹¹ have also demonstrated amino acid synthesis by the long-wavelength UV irradiation of two carbon atom precursors photosensitized by H_2S . Both reports indicate quantum yields of the order of 10^{-5} . The apparent quantum yields we report here ($\sim 2.4 \times 10^{-5}$) are based on incident UV 254-nm photons and are uncorrected for absorption, reflection and shielding and are not directly comparable with those of Sagan *et al.*¹¹ or of Hong *et al.*¹⁰. Although no specific photosensitizers, such as H_2S or Hg, were present in our system, organic microstructures themselves absorb UV and visible light effectively (0.05 mg dry wt. has an $A_{254\text{nm}}$ of ~ 2.5). Presumably it is on the surfaces of these structures where photosensitization, perhaps to form free radicals¹², occurs. Tyson and Oyama¹³ demonstrated incorporation of gaseous $^{14}\text{CO}_2$ into UV photolysis products of solid amino acids at wavelengths >220 nm with apparent quantum yields of about 4×10^{-6} to 4×10^{-7} . Apparently aliphatic amino acids form free radicals when irradiated at 254 nm, which might then react with CO_2 (ref. 13). Hubbard, Hardy and Horowitz¹⁵ and Tseng and Chang¹² showed the production of organic compounds, principally formaldehyde and glycolic acid, on irradiation over a broad spectral range below 300 nm in surface catalysed reactions of CO , CO_2 and H_2O .

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Structurally preserved angiosperm flowers from the Upper Cretaceous of southern Sweden

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The angiosperms, or flowering plants, are believed to have originated and diversified during the Cretaceous. The earliest angiosperm fossils are pollen and leaf impressions found in late early Cretaceous strata^{1,2}. We now describe the discovery of fossil flowers in the Upper Cretaceous of Sweden which represent a significant addition to our very limited knowledge of the floral structure of Cretaceous angiosperms.

Among morphological and stratigraphic studies of the early angiosperm record^{1,3-6}, the investigations by Doyle and Hickey^{1,3} on the pollen and leaves from the Potomac Group of eastern North America have provided critical evidence on the early diversification of the group. Angiosperm reproductive structures in the Cretaceous are mainly fruits and seeds, and floral structures are extremely rare⁷⁻⁹, with the result that work on evolutionary relationships within the angiosperms^{10,11} has largely concentrated on living plants. Most Cretaceous flowers so far described are impression or compression fossils⁹ which usually yield only incomplete information of floral structures and arrangement of parts. Stopes and Fujii¹² described a petrified gynoecium from the late Cretaceous of Japan, but the perianth parts of the flowers were incompletely preserved and the organization of this structure, attributed to the Liliaceae, is not clear. The only other structurally preserved flower known to be from the Cretaceous¹³ was found in North America. This is preserved as a three-dimensional compaction and rather detailed information on the floral structure has been obtained, although the degree of compression limits the extent to which its organization can be reconstructed.

Our discovery of angiosperm flowers from Sweden represents the best-preserved Cretaceous floral structures available. The flowers were sieved from kaolin-rich sediments collected in Höganäs AB's quarry at Åsen, north-east Scania, Sweden, together with other fossil plant remains. They are usually found in cross-bedded, poorly sorted sands and their orientation along the sedimentary structures indicates transportation together with the mineral grains. The deposits are dated on palynological evidence as Upper Santonian or Lower Campanian; the flowers must be of about the same age. The sediments are considered to be of fluvial origin deposited close to the Senonian shoreline^{14,15}. The fossil flowers are a minor part of the organic remains obtained from the sieving, the commonest of the fossils

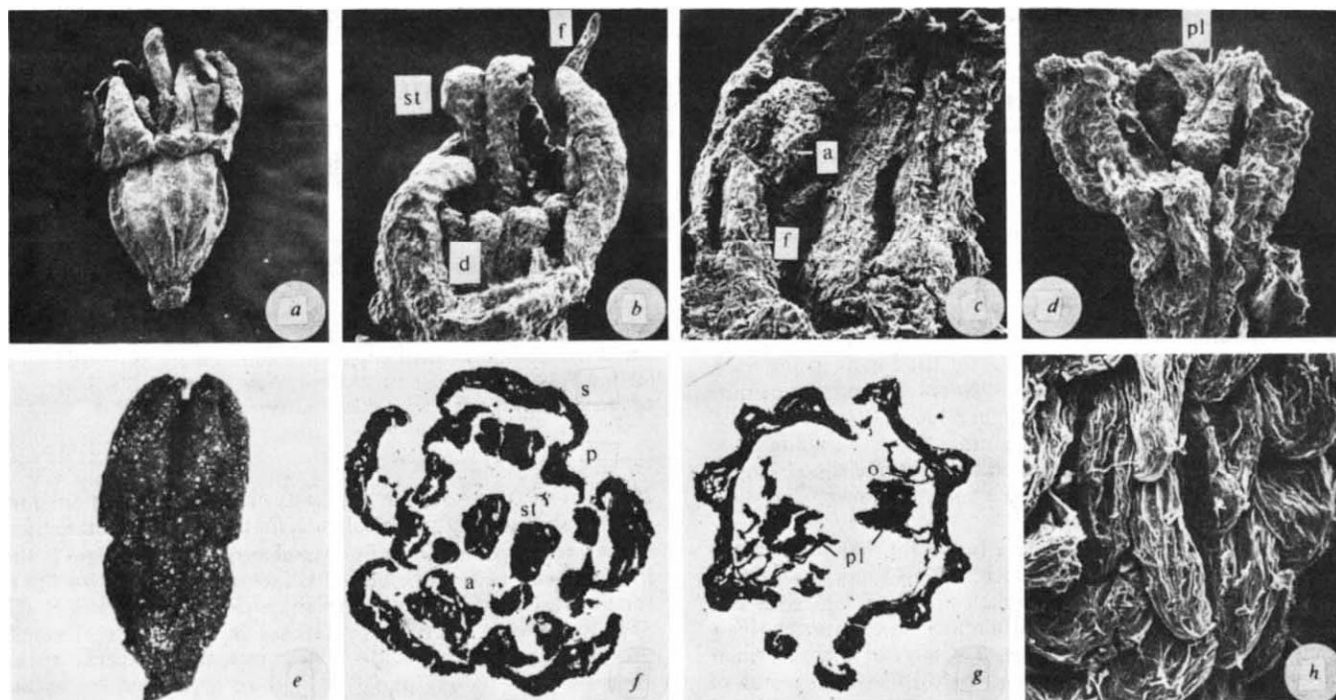


Fig. 1 Fossil flowers from the Upper Cretaceous of Scania, southern Sweden. *a*, Scanning electron micrograph of mature flower with one sepal missing, showing fragment of petal and filament ($\times 21$). *b*, Fragment of flower showing prominent disk (*d*) and styles with capitate stigma (*st*); the apex of one filament (*f*) projects beyond the sepals ($\times 60$). *c*, Part of flower bud with perianth removed, showing stamen with filament (*f*) and anther (*a*) preserved ($\times 110$). *d*, Fragment of ovary showing apical and lateral placenta (*pl*) ($\times 55$). *e*, Flower bud showing sepals and inferior ovary ($\times 28$). *f*, Transsection of flower bud shown in *e* with whorls of sepals (*s*), petals (*p*), anthers (*a*) and styles (*st*) ($\times 60$). *g*, Transsection through inferior ovary showing two placenta (*pl*) and ovules (*o*) ($\times 60$). *h*, Ovules attached to the placenta, removed from the ovary ($\times 230$). The specimens will be deposited at the Department of Geology, University of Stockholm, Sweden.

being fruits and seeds. Conifer leaves of unknown affinity are common in some samples, and megaspores, sporangia and dispersed anthers have also been recovered, although the angiosperm remains are the dominant element. Palynological studies^{16,17} show that the angiosperms are also predominant among the palynomorphs, and pollen forms referred to the Normapolles complex are common.

One flower type represented by several developmental stages from small flower buds to mature flowers (Fig. 1*a, e*) has been examined in detail using the scanning electron microscope, as well as by serial sectioning for light microscopy. The flowers are preserved in a three-dimensional state, which we attribute to their charcoal composition. Thin sections of the tissue show homogenization of the cell wall, a feature which is considered to be characteristic for fusinite (charcoal)¹⁸. Charring probably occurred within the litter at the soil surface as a result of forest fire, before transport and incorporation in the sediment. Such transformation to charcoal evidently confers rigidity and resistance to collapse on burial and several records of relatively delicate plant parts preserved three-dimensionally in charcoal are now known^{19,20}.

The fossil flowers are ~ 2 mm long and 1 mm broad. From the degree of opening of the perianth, most of the flowers must have been close to maturity, even though so minute. All floral parts except the anthers seem to be persistent so that examination of the androecium was restricted mainly to the flower buds. The flowers are regular and bisexual with radial symmetry (actinomorphic) and with the floral parts in whorls. The sepals seem to be more resistant than the petals and are more frequently preserved. Both sepals and petals number five and are free (Fig. 1*f*), the calyx being imbricate and the corolla of open aestivation. The androecium is apparently diplostemonous and composed of 10 stamens. The anthers are dorsi-fixed and

introrse with longitudinal dehiscence (Fig. 1*c*). The pollen grains are slightly prolate, tricolpate, and very small ($\sim 10 \mu\text{m}$ in equatorial diameter), with a tectate exine. They are probably not mature.

The gynoecium is inferior with a unilocular ovary formed by two fused carpels. This inferior ovary is characterized by 10 longitudinal ribs (Fig. 1*a, e*). The gynoecium is surmounted by a prominent 10-lobed disk (Fig. 1*b*) which is thought to be a nectary; because of this, and on account of the floral structure generally, the flowers are believed to have been pollinated by insects. The ovary has two free styles, each terminated by a small capitate stigma (Fig. 1*b*). The fruit is a capsule splitting apically between the carpels and there are two apical and lateral, pendant placenta each with many ovules (Fig. 1*d, g, h*). The

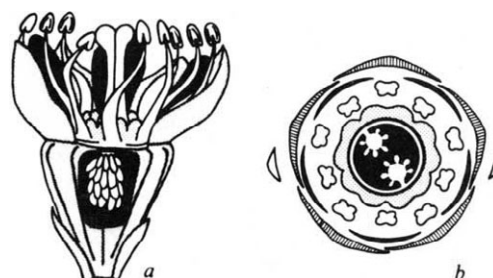


Fig. 2 Simplified reconstruction of the fossil flower (*a*), with part of ovary wall removed to show one placenta and floral diagram (*b*).

seeds are small and thin-walled with slightly elongated surface cells. The arrangement of the floral parts is shown in Fig. 2a, b.

The organization and structure of the fossil flower suggest a close relationship to a number of Recent genera of the Saxifragales. Diplostemonous flowers are known within the Saxifragales, although obdiplostemony or haplostemony is the common organization of the androecium. Unilocular and inferior ovaries are also found in a number of saxifragean families. Free, pendant placentae, however, are not common, and have only been observed in a few genera. Although all characters known to occur in the fossil flower are found within the saxifragean complex, no modern genus seems to possess exactly the same combination of characters as the fossil flower. However, it seems appropriate to refer the fossils to the Saxifragales and it is believed that the flowers may be close to the source of several modern saxifragean lines. Among the fossil plant remains from our material another flower with affinity to the Saxifragales has been discovered. It is distinguished from the above described flower in having a bilocular ovary and axile placentation.

The fossil flowers described here represent the oldest epigynous flower known in the fossil record. This report also documents the presence of the Saxifragales in the Cretaceous and demonstrates that the group had already reached a relatively high level of evolution, with syncarpous, inferior and unilocular gynoecia. Although the attainment of the major features of angiosperm diversity within the Cretaceous is suggested by leaf and pollen characters^{1,3}, this is the first demonstration of these particular advanced floral features within the Cretaceous.

The Saxifragales are represented in the Tertiary by a number of genera and by the end of the Eocene the presence of four families, the Itaceae, Pittosporaceae, Hydrangeaceae and Saxifragaceae, have been documented by the occurrence of floral structures from the Baltic Amber²¹. Our discovery of saxifragean flowers in the Cretaceous thus demonstrates that some of the diversity of the group seen in the Lower Tertiary was already evident in the Cretaceous. This is consistent with the central position of the Saxifragales in angiosperm phylogeny postulated by Takhtajan¹⁰ and Hutchinson¹¹.

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Congruent shifts in sand eel abundance in western and eastern North Atlantic ecosystems

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It has been suggested, on the basis of model simulations and slowly accumulating empirical data, that changes in the structure of marine ecosystems may be caused as much by changes in the trophic levels as by environmental factors^{1,2}. Support for this is found in recently observed shifts in species abundance of North Sea fish stocks, where large catches in the 1970s of small, fast-growing, opportunistic plankton-feeding fishes—sprat, sand eel and Norway pout—have been accounted for as the result of the replacement by these species of depleted herring and mackerel stocks^{3,4}. This suggestion is difficult to confirm, as knowledge of the abundance of these 'opportunistic' species since the mid-1960s is largely limited to catch data for the North Sea⁵. The only sand eel report independent of commercial fisheries catch data is for larvae collected in 1948–68, just before the sharp decline in mackerel and herring stocks⁶. The area surveyed did not, however, cover all the major population centres in the North Sea. In support of replacement, we describe here evidence from the north-west Atlantic which indicates that population explosions of small, fast-growing sand eel can coincide with depletions of larger tertiary predators, including herring and mackerel in a continental shelf ecosystem. Sand eels serve as an important link in marine food chains, preying on secondary producers and constituting an important food of higher trophic level fish and marine mammals^{7,8}.

Our results are based on 6 years (1974–79) of ichthyoplankton surveys, conducted by the United States, Poland and the Soviet Union on the continental shelf off the east coast of the United States, as part of a joint MARMAP study of shelf productivity. [MARMAP is the first multinational programme for measuring long-term changes in key structural components of very large marine ecosystems. At present 260,000 km² of the north-west Atlantic continental shelf are being sampled routinely to measure changes in key structural components, including primary production (¹⁴C), chlorophyll a phaeophytin, nutrients (NO₂, NO₃, SiO₃, NH₄ and PO₄), zooplankton, ichthyoplankton, fish, benthos, seabirds, water-column temperature, salinity and circulation⁹.] Between 6 and 12 surveys were done per year. The sampling area covered the continental shelf and included four sub-areas: Gulf of Maine, Georges Bank, southern New England and middle Atlantic Bight (see Fig. 1). Ichthyoplankton were collected at about 180 locations situated 25–35 km apart and during the 6 yr, we sampled ~6.5 million km².

The data presented here are based on population densities of sand eel calculated for the annual spawning period in winter, when sand eel larvae, *Ammodytes* spp., are most abundant. Two species, *A. dubius* and *A. americanus*, reportedly occur in the survey area¹⁰. Their taxonomy remains confused and we are uncertain whether our collections included only one or both. Thus, until the identity of the larvae is clarified, they are classified as *Ammodytes*.

At each sampling location, tows for ichthyoplankton and zooplankton were made through the water column using a paired bongo-type plankton sampler¹¹ with 60-cm openings and

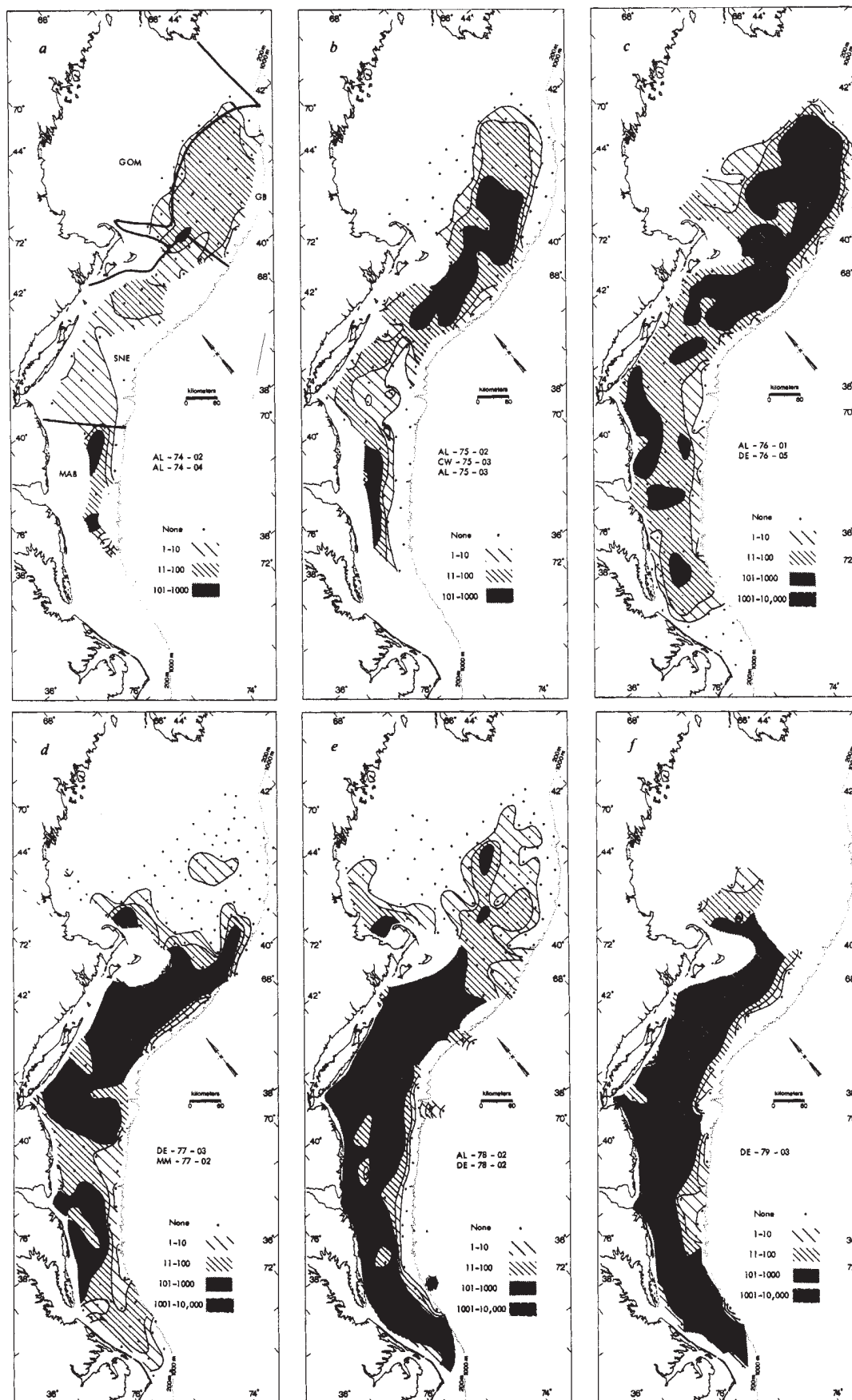


Fig. 1 Increase in abundance of sand eel larvae (*Ammodytes*) on the continental shelf off the north-east United States coast in winter 1974-1979. The four sub-areas of the shelf sampled include the Gulf of Maine (GOM), Georges Bank (GB), southern New England (SNE) and mid-Atlantic Bight (MAB). Samples were collected from the NOAA research vessels: Albatross IV (AL), Delaware II (DE), Commonwealth (CW) and Mount Mitchell (MM). *a*, 11 February-22 March 1974; *b*, 12 February-16 March 1975; *c*, 10 February-24 March 1976; *d*, 12 February-8 April 1977; *e*, 14 February-17 March 1978; *f*, 23 February-15 March 1979. Shading represents number of *Ammodytes* larvae per 10 m² surface area.

Table 1 Winter abundance estimates of sand lance, *Ammodytes*, larvae in three sub-areas off northeastern United States

Year		Georges Bank (41,809 km ²)	Southern New England (59,906 km ²)	Middle Atlantic (58,326 km ²)	Total ($\times 10^{10}$)
1974	<i>k</i>	33.941	11.216	48.692	49.309
	s.e.	1.054	1.093	1.726	
	Abundance	14.190	6.719	28.400	
	% Of larval fish	55	13	88	
1975	<i>k</i>	90.427	51.555	57.044	101.961
	s.e.	1.602	1.220	1.371	
	Abundance	37.806	30.884	33.271	
	% Of larval fish	59	60	90	
1976	<i>k</i>	1,018.311	243.798	55.393	604.105
	s.e.	1.990	1.440	1.297	
	Abundance	425.746	146.050	32.309	
	% Of larval fish	97	96	84	
1977	<i>k</i>	9.931	603.255	76.991	410.443
	s.e.	1.996	1.446	1.404	
	Abundance	4.152	361.386	44.905	
	% Of larval fish	88	81	71	
1978	<i>k</i>	13.916	569.359	313.297	529.631
	s.e.	1.122	1.451	1.332	
	Abundance	5.818	341.080	182.733	
	% Of larval fish	91	92	94	
1979	<i>k</i>	442.324	738.239	577.487	964.006
	s.e.	1.934	1.402	1.561	
	Abundance	184.931*	442.250	336.825	
	% Of larval fish	98	79	87	

Abundance estimates were determined by Δ -distribution²⁵. Retransformed mean abundance (*k*) is expressed as larvae per 10 m² surface area. Larval abundance ($\times 10^{10}$) is expansion of *k* to reflect the sub-area size. (See Berrien *et al.*²⁶ for discussion of rationale and procedures for use of Δ -distribution.)

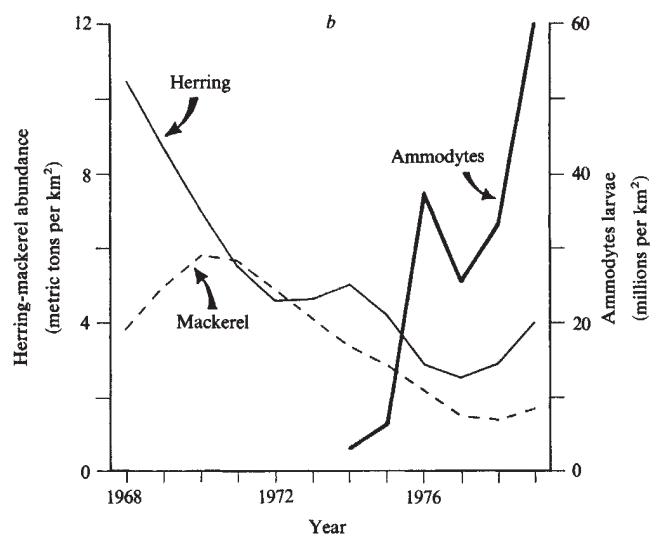
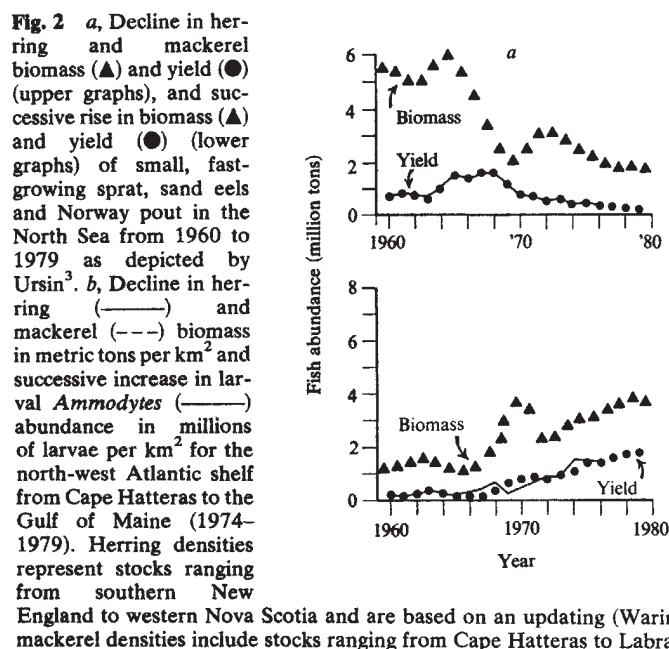
* Estimate based on limited sampling.

nets of 0.333 and 0.505 mm mesh. These nets were towed at ship speeds ranging from 1.5 to 3.5 knots. The bongo sampler was lowered obliquely from the surface to within 5 m of the bottom at a wire speed of 50 m min⁻¹ and retrieved at 20 m min⁻¹. Water filtered through the net was measured with a flow meter and a time-depth recorder was used to measure the towing path of the sampler. The ichthyoplankton samples were sorted, species identified and enumerated, and measured at the Plankton Sorting Center, Szczecin, Poland. The data on ichthyoplankton were calculated initially as numbers of larvae per 10 m² of sea surface, and expanded to numbers of larvae per km² and numbers of larvae $\times 10^{10}$ for the entire shelf.

The north-west Atlantic ecosystem off the northeastern United States has been subjected to significant fishing stress; fish biomass in the region was reduced by 50% from 1968 to 1975

(ref. 12). Since then, the silver hake and squid stocks have been increasing but herring and mackerel stocks remain low¹³. From 1974 to 1979, coincident with the low mackerel and herring stocks, we observed a population explosion of sand eel larvae increasing from a low shelf density of 49×10^{10} in 1974 to 964×10^{10} in 1979. During this 6-yr period the percentage of sand eel increased from less than 50% of the total mid-winter ichthyoplankton community to more than 85%. A summary of comparisons of abundance levels and percentage composition of sand eel in the ichthyoplankton community among areas and in years is made in Table 1.

The centre of sand eel larval abundance shifted during the years of study. Abundance was low in 1974 and 1975. In 1976 the centre of population density was on Georges Bank. Abundance estimates were significantly lower on Georges Bank in



1977 and 1978 but larvae of other taxa were also greatly reduced in number and the percentage contribution of sand eels remained close to 90%. Because of technical difficulties we were unable to complete our early winter survey in 1979. After 1976, the principal centres of abundance were in the southern New England and mid-Atlantic Bight areas; large patches of larvae in densities of over 1,000 per 10 m² extending for distances greater than 300 km occurred most frequently off the southern New England coast (Fig. 1).

In the North Sea, the fishery yield for juvenile and adult sand eel, sprat and Norway pout increased from <200,000 metric tons in 1960 to 1.6 million metric tons in 1976. In the absence of a fishery in the north-west Atlantic, it is difficult to estimate the contribution of larval abundance of sand eel to the biomass of adults. Based on available evidence, we conclude that the adult stock is increasing. (1) MARMAP bottom trawl surveys have shown an increasing trend in abundance in southern New England and the mid-Atlantic Bight¹⁴. (2) An increase in abundance has been observed directly by divers¹⁵, and (3) a significant increase has been observed in the consumption of sand eel by predators from 1968 to 1976 (ref. 16).

The influence of temperature has been suggested as a principal factor in controlling abundance levels of fish stocks on the continental shelves of the north-west Atlantic off the Canadian maritimes and the northeastern United States^{17,18}. The reductions in herring and mackerel on both sides of the Atlantic in response to heavy fishing mortality, followed by increases in sand eel and other small, fast-growing fish as shown in Fig. 2a, b make unlikely the hypothesis that the changes are due to environmental factors. Our findings show that when a large biomass of mid-size predators is removed it can be replaced by smaller, faster-growing, opportunistic species as described for the North Sea^{3,4} and observed by us.

The full consequence of structural change with respect to earlier unperturbed status has not yet been documented for large marine ecosystems. We are just starting to obtain the multitrophic level data needed to unscramble inter- and intra-specific ecosystem relationships^{9,19-22}. The influence of structural changes on aquatic ecosystems can result in significant economic and aesthetic loss. In a species succession model for the Great Lakes, removal of large predators resulted in the proliferation of fast-growing smaller alewives²³. A size-component response to structural modification in a large marine ecosystem has been observed in the Antarctic where, based on recent population trends²⁴, the decrease in the biomass of large blue whales may be in the process of replacement by the faster-growing smaller minke whales and seals. Monitoring of the shelf ecosystem in the north-west Atlantic is continuing in an attempt to measure the impact of any increase in the demersal and pelagic fish-predator field (for example, cod, haddock, hake, mackerel and herring) on the changing structure of the shelf ecosystem.

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Monocyclic β -lactam antibiotics produced by bacteria

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A recent article¹ described two novel monocyclic β -lactam antibiotics produced by bacteria. In the past few years, we have screened bacteria isolated from numerous ecosystems and habitats for their ability to produce β -lactam antibiotics, using a strain of *Bacillus licheniformis* which is specific for molecules containing a β -lactam and which is sensitive down to 100 ng ml⁻¹. From over one million bacterial isolates screened, we have now identified seven related molecules containing β -lactams produced by a range of bacterial species. For this new family of monocyclic β -lactams we suggest the class name 'monobactams'. Bacteria which produce β -lactams are, in reality, relatively common in nature.

The β -lactam most frequently detected in our studies was SQ 26,445 (Fig. 1, I), one of the compounds isolated from a strain of *Pseudomonas acidophila*¹. β -Lactam-producing strains isolated from 41 separate locations were identified as species of

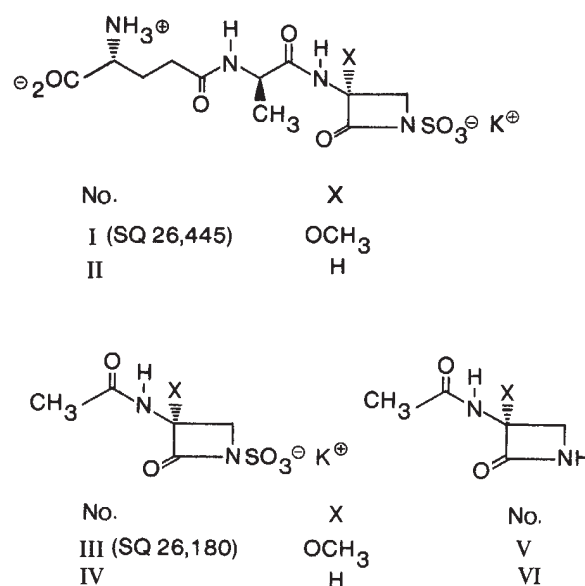


Fig. 1 Structures of monobactams I-VI. SQ 26,445 (I) was isolated from a strain of *Gluconobacter*. SQ 26,180 (III) was produced by strains of *Chromobacterium violaceum*.

Table 1 Antibacterial activity of monobactams

Organism	SC	I	MIC ($\mu\text{g ml}^{-1}$)	
			III	IV
<i>Staphylococcus aureus</i>	1,276	>100	50	50
<i>Escherichia coli</i>	10,896	25	25	>100
<i>Klebsiella pneumoniae</i>	9,527	50	>100	>100
<i>Proteus mirabilis</i>	3,855	12.5	>100	>100
<i>Enterobacter cloacae</i>	8,236	25	>100	>100
<i>Pseudomonas aeruginosa</i>	9,545	50	3.1	>100

Minimum inhibitory concentrations (MICs) were determined by a twofold agar dilution method on DST agar (Oxoid). Final inoculum level was 10^4 colony-forming units.

Gluconobacter or *Acetobacter*, and the original producing strain of SQ 26,445 as a polar-flagellated, oxidative Gram-negative rod. This culture was cytochrome oxidase negative, positive for catalase, citrate and esculin hydrolysis and accumulated poly- β -hydroxybutyric acid. Growth was good at pH 4.5 in malt yeast extract medium; acid production was observed as a zone of clearing around colonies grown on yeast extract/glucose/calcium carbonate agar. The organism was assigned to the genus *Gluconobacter* on the basis of these characteristics.

For isolation of SQ 26,445 fermentations were carried out at 25 °C for 18–24 h with aeration and agitation in a 380-l fermenter containing 250 l of medium (0.5% yeast extract and 1.0% glucose in distilled water). SQ 26,445 was purified by a combination of ion-exchange, charcoal, and reverse-phase chromatography. The structure (Fig. 1, I) was assigned by amino acid analysis of hydrolysis products (6 M HCl), N-terminal analysis² and NMR spectroscopic comparison to γ -Glu-Ala³ and SQ 26,180 (*vide infra*).

The simplest monobactam found was SQ 26,180 (Fig. 1, III) produced by strains of *Chromobacterium violaceum*. Although many thousands of *C. violaceum* strains were examined, organisms producing III were isolated from only eight locations; each of these was identified as *C. violaceum* on the basis of the following characteristics. The organism was an aerobic Gram-negative rod, motile due to a single polar flagellum with an occasional single smaller lateral flagellum. Colonies of this bacterium gave a violet pigment on nutrient agar and production of pigment was enhanced by the addition of either tryptophan or yeast extract. Good growth was observed between 15 and 37 °C,

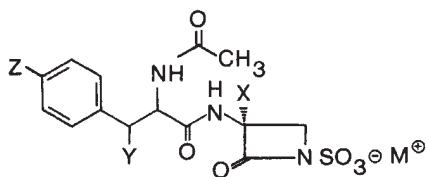
with no growth below 4 °C or above 37 °C. Casein was hydrolysed strongly and esculin hydrolysis was negative. Glucose, fructose and trehalose were fermented; L-arabinose was not used fermentatively or oxidatively; HCN was produced.

For isolation of SQ 26,180, fermentations were carried out at 25 °C for 18–24 h with aeration and agitation in a 380-l fermenter containing 250 l of medium (yeast extract, 0.25%; glucose, 3.0%; N-Z amine-A, 1.0% in tap water). SQ 26,180 was concentrated from broth by ion-pair extraction and purified by partition, ion-exchange, size-exclusion and reverse-phase chromatography. The structure (Fig. 1, III) was assigned by spectroscopic deduction and confirmed by synthesis from azetidinone V (refs 4, 5) which also demonstrated the absolute

Table 2 Susceptibility to and inhibition of β -lactamases

Compound	Enzyme	Relative		
		$V_{\max}$	K_m (mM)	I_{50} (mM)
Benzylpenicillin III IV VIII IX	<i>S. aureus</i> (PCI)	100	0.03	—
		<0.01	—	>2.0
		0.71	0.04	—
		<0.02	—	>0.4
		12	0.98	—
Cephaloridine III IV VIII IX	<i>E. coli</i> (TEM-2)	100	0.66	—
		<0.01	—	>2.0
		12	0.68	—
		<0.01	—	>0.4
		10	0.28	—
Cephaloridine III IV VIII IX	<i>Klebsiella</i> (K-1)	100	0.15	—
		<0.04	—	>2.0
		86	0.45	—
		<0.03	—	>0.4
		220	1.3	—
Cephaloridine III IV VIII IX	<i>Enterobacter</i> (P-99)	100	0.58	—
		0.06	—	0.22
		0.46	0.41	—
		<0.01	—	0.002
		0.15	0.19	—

All studies were performed at 25 °C in 0.1 M phosphate buffer, pH 7.0, using homogeneous TEM-2, P-99 and *S. aureus* β -lactamases. K-1 β -lactamase was a partially purified preparation containing a single β -lactamase activity. Monobactams were prepared in 20 mM KH_2PO_4 and diluted in 0.1 M phosphate buffer before assays. β -Lactamase activities were determined using a Gilford 250 spectrophotometer at the following wavelengths: 295 nm for cephaloridine; 240 nm for benzylpenicillin; 206 nm for compounds III, IV and VIII and 235 nm for IX. Kinetic constants were obtained from Lineweaver-Burk plots of at least four concentrations for each substrate. Relative $V_{\max}$ values were calculated by setting $V_{\max}$ for cephaloridine or benzylpenicillin to 100. I_{50} values, the concentration of compound required to inhibit enzyme activity by 50%, were determined using 1.0 mM cephaloridine as the substrate for P-99 β -lactamase and 0.5 mM benzylpenicillin as the substrate for TEM-2, K-1 and *S. aureus* β -lactamases.



No.	X	Y	Z	M
VII	OCH_3	H	H	Na
VIII	OCH_3	H	OH	K
IX	H	H	OH	K
X	OCH_3	OH	$\text{OSO}_3^- \text{Na}^+$	Na
XI	OCH_3	$\text{OSO}_3^- \text{Na}^+$	$\text{OSO}_3^- \text{Na}^+$	Na

Fig. 2 Structures of monobactams VII–XI, produced by *Agrobacterium radiobacter*.

configuration. Thus, treatment of V with dimethylformamide- SO_3 complex, followed by workup with buffered tetra-*n*-butyl ammonium bisulphate, gave the tetra-*n*-butyl ammonium salt of III, which was identical to material prepared from crystalline SQ 26,180, melting point 194 °C (dec). The unmethoxylated analogue IV was similarly prepared from azetidinone VI (ref. 5).

Agrobacterium radiobacter SC 11,742 produces a mixture of monocyclic β -lactams, five of which have been identified as VII–XI (Fig. 2). Compound IX is the first naturally occurring non-methoxylated derivative isolated in quantity.

The β -lactam-producing organism was identified as *A. radiobacter* on the basis of the following. It was an aerobic Gram-negative rod, motile by means of sub-polar and peritrichous flagella. The strain was cytochrome oxidase positive,

arginine dihydrolase negative and produced 3-ketolactose from lactose⁶. Growth of the organism on mannitol yeast extract agar containing Congo red resulted in dye uptake⁷. Fluorescence was not observed, and there was no pathogenicity for abraded stems of tomato and sunflower seedlings.

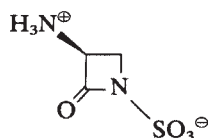
For isolation of compounds VII–XI, fermentations were carried out at 25 °C for 40–45 h with aeration and agitation in a 380-l fermenter containing 250 l of medium yeast extract (0.5% and glucose, 1.0% in distilled water). The mixture of VII–XI was isolated from broth by ion-pair extraction. Chromatography on Sephadex G-10 in water gave rough separation according to charge type (XI eluted first, followed by X and finally a mixture of VII–IX). Further purification by combinations of ion-exchange and HP-20 chromatography gave pure samples of VII–XI. Structures were assigned to these by elemental composition and spectroscopic properties. The structures of IX and II were confirmed by synthesis from 6-aminopenicillanic acid (6-APA) (to be described elsewhere). Our assignments of C-3 absolute configuration in I, VII, VIII, X and XI rest on the analogy with III; side-chain absolute configuration has only been rigorously determined for I and IX (D-Tyr). Compound IX is thought to be the precursor of VIII (ref. 8); indeed, we have detected small quantities of II and IV by HPLC in the fermentation broths from which we isolated I and III.

Antimicrobial activities of selected monobactams are shown in Table 1. Although the compounds are weakly active against a range of bacteria, the methoxylated compound III shows greater activity against Gram-negative organisms, especially *Pseudomonas*, compared with the non-methoxylated derivative IV. Compounds VII–XI showed similar antimicrobial properties to those observed for compounds III and IV.

The methoxylated monobactams III and VIII showed a high degree of stability to β -lactamases from both Gram-positive and Gram-negative organisms, which agrees with previous work¹, whereas the desmethoxy monobactams IV and IX were readily hydrolysed by enzymes with primarily penicillinase activities. The *Enterobacter* P-99 cephalosporinase was the only enzyme in this series to show significant affinity for all the monobactams tested (Table 2).

The intrinsic activity of III and IV was assessed on the basis of binding to essential penicillin binding proteins (PBPs) in *Escherichia coli*. The PBP profile indicated a lack of affinity (up to 100 $\mu\text{g ml}^{-1}$) for any of the essential PBPs. Interestingly, methoxylation of the monobactam nucleus increased binding to the non-essential PBP 4 and 5/6 (D, D-carboxypeptidase IB and IA, respectively)^{9,10}, an effect similar to that observed for cephalosporins.

On the basis of the novel nucleus XII (3-aminomonobactamic acid, 3-AMA) we have synthesized a highly active β -lactamase-stable derivative which has been developed for clinical evaluation and will be reported elsewhere.



XII (3-AMA)

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Vasopressor receptor antagonist prevents behavioural effects of vasopressin

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The antidiuretic hormone vasopressin¹ also has a direct vasoconstrictor effect on the smooth muscles of the vascular system². This may be physiologically significant³, but higher doses of the hormone are required in conscious subjects than when the autonomic nervous system is depressed by anaesthesia or ganglionic blocking agents². Vasopressin may also function as a releaser of corticotropin^{4,5}, in the maintenance of learned avoidance behaviours and in memory consolidation^{6–8}. Although these functions are controversial^{9–11}, it is of interest that corticotropin^{12,13} and other peptides cleaved from the proopiomelanocortin¹⁴ precursor also influence avoidance behaviours¹³. Here, we have examined a highly potent peptide analogue¹⁵ of arginine vasopressin (AVP) for its ability to antagonize the pressor and behavioural responses to AVP in conscious rats. Our results indicate that doses of AVP which prolong extinction of active avoidance behaviour for several hours after subcutaneous injection^{8,13,16}, also produce early pressor responses. Conversely, we also find that doses of the antagonist peptide, 1-deaminopenicillamine, 2-(O-methyl)tyrosine AVP (dPTyr(Me)AVP), which can prevent the pressor response¹⁵, also abolish the effects of subcutaneously injected AVP on prolongation of extinction. This blockade indicates that signals from peripheral visceral sources may have an important role in the subsequent behavioural changes, and suggests that the receptors at which AVP elicits its pressor effect are similar to those leading to its behavioural action.

For measurements on blood pressure in conscious animals, Wistar rats (Charles River Farms, 300–400 g) were anaesthetized with halothane through an open face mask. The abdominal aorta was cannulated with PE 10 polyethylene tubing through the left femoral artery, and tied in place about 2.5 cm above the entrance to the abdominal cavity. The tubing, filled with heparinized saline (20 units ml^{-1}), was then plugged distally and led through the subcutaneous space to exit through a dorsal incision at the inter-scapular level. Cannulae were flushed daily with heparinized saline and remained patent for at least 5 days. Beginning 24 h or more after cannulation, each rat was placed in a sound-attenuated experimental chamber and the cannula connected to a Statham pressure transducer (P2310) and monitored with a Buxco (Quincy) cardiovascular analyser for heart rate and systolic, diastolic and mean arterial pressure. Each rat was used only once a day. Blood pressure was monitored at various times after subcutaneous injection of saline or various doses of AVP or dPTyr(Me)AVP.

Arginine vasopressin was synthesized at the Salk Institute (J.R.). The vasopressin analogue dPTyr(Me)AVP was synthesized first at the Medical College of Ohio (M.M., W.H.S.) and subsequently at the Salk Institute (J.R.). This compound has been fully characterized as a specific antagonist to the pressor actions of vasopressin in the urethane-anaesthetized rat¹⁵; in

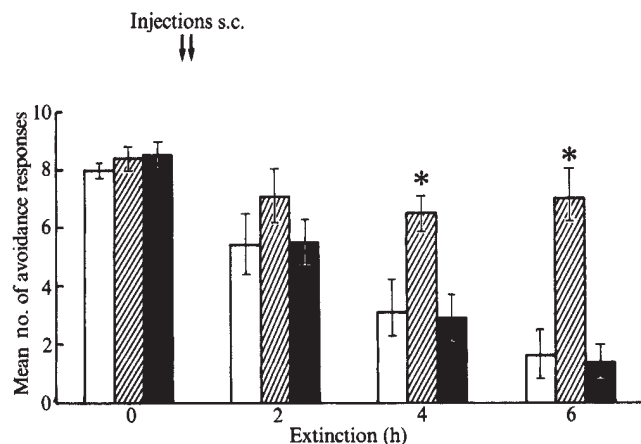


Fig. 1 Effects of AVP and AVP plus dPTyr(Me)AVP on extinction of active avoidance behaviour. After 3 days of training, rats meeting the criteria of at least seven successful avoidances during the first 10 trials of extinction were injected subcutaneously (s.c.) with saline (twice at 2.0-min intervals), AVP plus saline or AVP plus dPTyr(Me)AVP immediately after the first set of 10 extinction trials on day 4. Rats receiving AVP only show persistent avoidance throughout the 6 h of observation, when tested on 10 trials at each of the three next 2-h intervals, whereas rats receiving either saline with no peptide or both peptides extinguished this active avoidance behaviour at similar rates. □, Saline+saline ($n=9$); ▨, saline+AVP, 6 μg per kg ($n=9$); ■, dPTyr(Me)AVP, 30 μg per kg+AVP, 6 μg per kg. *Significantly different from both the saline with no peptide and both peptides groups; $P < 0.05$ Newman-Keuls test following analysis of variance.

this preparation AVP is a more potent pressor agonist than in unanaesthetized rats². Before injection, peptides were first dissolved in 50 μl of 0.01 M hydrochloric acid and then further diluted in 0.9% NaCl.

For behavioural experiments rats (140–160 g) were handled for 30 s for 2–5 days after arrival in the laboratory. They were trained and tested in a modified Skinner box (BRS/LVE) 25 cm $\times$ 30 cm $\times$ 27 cm containing a grid floor, composed of 16 bars, 0.5 cm in diameter and spaced 2 cm apart, for administering shock. A wooden pole, blackened with ink and 1.67 cm in diameter, extended 25 cm from the cage floor to the ceiling; a 50-W incandescent light bulb was centred just above the clear Plexiglass ceiling and served as the conditioned stimulus.

For acquisition, each rat was subjected to 10 trials per day for 3 days. The inter-trial interval was 40, 60 or 80 s, with an average of 60 s. A trial began with illumination of the conditioned stimulus followed 5 s later by a scrambled a.c. shock (0.3–0.7 mA) delivered to the grid floor, lasting up to 30 s or until the rat jumped on the pole. During the first three trials on the first day of acquisition, rats failing to jump on the pole within 5–10 s were gently placed on the pole by the experimenter. Throughout training, rats failing to climb down the pole within 30 s after jumping were gently turned around on the pole and thus forced to return to the grid floor. All testing was accomplished in a darkened room between 1000 and 1900 h. For extinction, the same procedure was used except that the shock was omitted and the rats were subjected to 10 trials every 2 h during the fourth day. Rats were selected for drug injections by a similar procedure to that reported by De Wied and associates⁸. Only rats that made seven or more avoidance responses during the first 10 extinction trials were treated with peptide or placebo immediately after the session and continued in the experiment through extinction trials. All injections were subcutaneous, in the neck, with a constant volume of 0.5 ml of peptide solution. Control rats received saline alone. All behavioural testing was performed according to a 'blind' protocol in which the experimenter testing the rats was unaware of the subject's treatment.

Results on systolic blood pressure or on extinction, for a given time point, were analysed with a single-factor analysis of vari-

ance (ANOVA). Subsequent individual mean comparisons were made using the Newman-Keuls test, but only at those time points showing a significant ANOVA value, that is, $P < 0.05$.

In confirmation of previously published observations by the De Wied group^{8,12,13} and our own¹⁶, subcutaneous injections of as little as 1 μg AVP ($\sim 6 \mu\text{g}$ per kg) gave reproducible prolongation of the extinction of active (pole jump) avoidance which was highly significant for as long as 6 h (see ref. 16 and Fig. 1). We therefore examined this dosage range for effects on arterial pressure in the conscious rat (Figs 2, 3), and found that both 5 and 25 μg per kg doses of AVP led to prompt elevations of arterial blood pressure without overt behavioural effects. These effects peaked at 10–20 min after injection and were generally within normal ranges after 1 h (data not shown). Using the threshold pressor dose of AVP at 5 μg per kg, we evaluated various doses of the antagonist peptide and found that the pressor response was significantly depressed and delayed with 5 μg per kg dPTyr(Me)AVP, and apparently completely blocked by a 25 μg per kg dose (Fig. 3). A 10-fold higher dose of the antagonist also produced a significant (but equally transient) lowering of basal systolic pressure (Fig. 2). As the 25 μg per kg dose of antagonist completely blocked the response to a 5 μg per kg dose of AVP but did not itself cause hypotension, the 5:1 ratio of antagonist to agonist was used in further tests of AVP in active avoidance extinction (Fig. 1); here, the co-injection of dPTyr(Me)AVP with AVP completely prevented the prolongation of extinction at 4 or 6 h after the injection. Rats receiving AVP and the antagonist therefore did not differ from saline-injected rats and showed normal extinction behaviour, whereas rats receiving AVP and saline showed markedly prolonged extinction, throughout the 6 h of testing.

Although the mechanism by which AVP and several other peptides alter extinction behaviour in active or passive avoidance paradigms is unknown, it has been hypothesized that such actions are centrally elicited because intracerebroventricular injections of even smaller amounts of these peptides produce these same behavioural effects. However, even with intracerebroventricular injections it is impossible to know whether

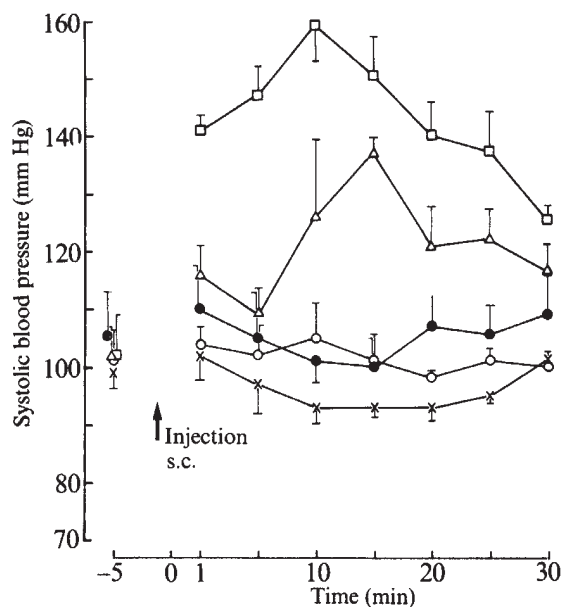


Fig. 2 Effects of AVP on systolic blood pressure in conscious rats, as recorded by direct pressure transduction through chronically implanted arterial cannulae before and after the subcutaneous injections indicated. After AVP doses of 5 or 25 μg per kg, blood pressures were rapidly and significantly elevated; 250 μg per kg doses of the antagonist peptide significantly lowered basal arterial pressure. □, AVP, 25 μg per kg ($n=3$); Δ, AVP, 5 μg per kg ($n=3$); ●, AVP, 1 μg per kg ($n=3$); ×, dPTyr(Me)AVP, 250 μg per kg ($n=3$); ○, saline ($n=3$).

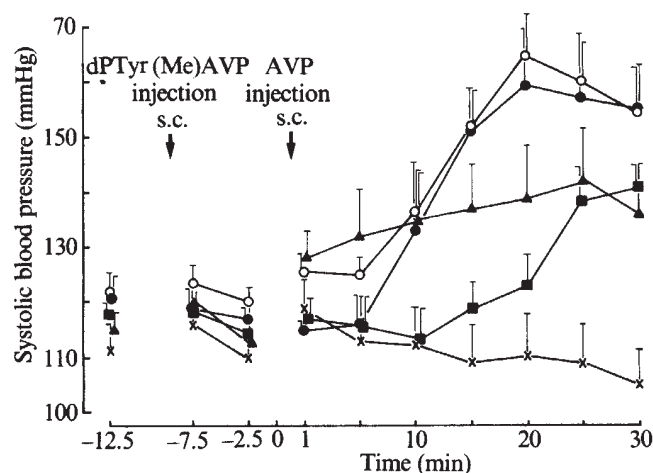


Fig. 3 Effects of dPyr(Me)AVP on increases of systolic blood pressure produced by AVP. The antagonist peptide at various doses was injected subcutaneously 7.5 min before the subcutaneous injection of a constant dose (5 µg per kg) of AVP. Compared with saline-injected controls ('0 µg per kg' antagonist), blood pressure responses were significantly depressed by 1 and 5 µg per kg doses of antagonist and completely antagonized by the 25 µg per kg dose. Doses of dPyr(Me)AVP (µg per kg): ○, 0 (n=3); ●, 0.3 (n=3); ▲, 1.0 (n=4); ■, 5.0 (n=6); ×, 25 (n=4).

the targets responsible for the altered behaviour are within the substance of the brain or within the ventricular spaces. Examples of the latter sort of target, which would be open to contact with circulating blood or extracellular space, are the circum-ventricular organ systems¹⁷. Although blood-brain barriers are reduced in some brain regions, such as hypothalamus and area postrema, these areas are not generally regarded as critical for memory processes. The present results provide additional clues to the possible sites and mechanisms of the peptide-induced behavioural alterations, as they indicate that in the conscious rat, doses of AVP injected peripherally elicit a significant rise in arterial pressure, and that when this pressor response is prevented by an analogue of AVP, the behavioural effects of AVP are also antagonized.

Our data could be interpreted to indicate that the vascular response, and perhaps other visceral effects, including evoked release of corticotropin^{4,5}, could lead to altered visceral afferent signals which are sufficient to alert the rat and to cause the persistence of ongoing or escape behaviours. For example, an acute induced hypertensive state can reduce reactivity to noxious stimuli through a baroreceptor-mediated reduction of cerebral arousal¹⁸. Under the latter view, the effects of AVP may be more generally interpreted as a hidden signal within a holistic set of integrative central nervous system visceral afferent information. Under extremes of dehydration or acute vascular hypovolaemia, such behavioural effects of AVP could be an important adjunct to hormone actions directed to restoration of fluid volume and general homeostasis³. Alternatively, the blockade of the behavioural response following blockade of the pressor response could simply indicate that AVP acts both centrally and peripherally and that the two events, pressor response and prolongation of extinction, are unrelated. In this view, even if the peripheral vascular effects of AVP are not directly the cause of the subsequent behavioural effects, it remains possible that the integrative actions of endogenous AVP as a physiological regulator elicit a state of central activation appropriate for stressful situations which are generated in parallel with the peripheral pressor action. Additional observations are therefore required to determine whether AVP and other peripherally injected peptides alter behaviour by directly activating central operational mechanisms such as memory or

retrieval or whether they induce altered behaviours by triggering peripheral receptors which in turn evoke some altered perception of visceral status that is sufficient to place the animal in a state of alerted surveillance.

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Chronic morphine administration increases the apparent number of α_2 -adrenergic receptors in rat brain

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When the α_2 -adrenergic agent¹ clonidine (Catapresan) is given to opiate addicts undergoing withdrawal, a rapid and dramatic reversal of objective signs and subjective symptoms of withdrawal can be observed², which include a marked decrease in hypertension and anxiety levels with no accompanying euphoria³. Such clinical findings have been correlated with noradrenergic neuronal activity in the locus coeruleus, and systemic or local administration of clonidine inhibits the firing of locus coeruleus noradrenergic cells⁴; administration of opiates produces a similar inhibiting effect. Stimulation of the locus coeruleus either electrically or pharmacologically (by the administration of α -adrenergic or opiate antagonists) increases the firing rate of these neurones⁵ and elicits many of the symptoms of opiate withdrawal⁶. Both morphine and clonidine have been shown to block the effects of electrical and pharmacological activation of the locus coeruleus in primates and rats⁴. However, single-unit recording techniques indicate that opiates and clonidine elicit their action at independent binding sites⁶⁻⁷, although they may activate a common mechanism. We have explored the possible relationship between opiates and clonidine at the receptor level, and demonstrate here that chronic, but not acute, administration of morphine to rats results in increased binding of clonidine to α_2 -adrenergic binding sites in brain. Increased binding of clonidine is due to an enhanced number of α_2 -adrenergic binding sites but no change in their affinity.

Male Sprague-Dawley rats (200-250 g) under light halothane anaesthesia were implanted subcutaneously with pellets containing 75 mg morphine sulphate (or control pellets). On the second day, two additional pellets were implanted⁸, and after

Table 1 Effect of chronic and acute morphine treatment on adrenergic binding

	Control specifically bound (fmol per incubation $\pm$ s.e.m.)	Treated	% Change
Chronic morphine			
Cortex			
^3H -clonidine	69.7 $\pm$ 3.0	93.2 $\pm$ 3.7*	+33.7
^3H -WB-4101	107.9 $\pm$ 3.4	102.7 $\pm$ 1.9†	-4.8
^3H -Dihydroalprenolol	29.2 $\pm$ 0.7	34.4 $\pm$ 1.9*	+17.9
Brain stem			
^3H -Clonidine	32.4 $\pm$ 2.1	48.2 $\pm$ 1.0*	+48.4
^3H -Dihydroalprenolol	22.8 $\pm$ 1.7	29.5 $\pm$ 0.8‡	+29.5
Acute morphine			
Cortex			
^3H -Clonidine	73.8 $\pm$ 3.9	70.5 $\pm$ 3.7†	-4
^3H -Dihydroalprenolol	32.6 $\pm$ 1.3	35.1 $\pm$ 1.6†	+7

Brains were weighed then homogenized in 40 vol (w/v) of ice-cold 0.05 M Tris-HCl buffer (pH 7.4 at 4°C). The homogenate was centrifuged at 18,000g for 20 min, the supernatant decanted and the pellet rehomogenized. After two such washes, the pellet was resuspended in 10 vol (original) of buffer and used in the assay. 1-[^3H] dihydroalprenolol (β -adrenergic receptors) and ^3H -WB-4101 (α -adrenergic receptors) binding were assayed as described earlier¹⁰⁻¹² except that 1 nM tritiated ligand was used. ^3H -clonidine binding (α_2 -adrenergic receptors) was determined by a modification of a published method^{10,11}. For the case of clonidine binding, 20 mg tissue weight equivalents of homogenate (200 μl) were used. Incubations were carried out at room temperature in 0.05 M Tris-HCl (pH 7.4 at 25°C) containing 2.0 nM ^3H -clonidine (NEN), and in early experiments the gift of Boehringer Ingelheim) in a total volume of 10.0 ml. Nonspecific binding is defined as that binding occurring in the presence of 10^{-5} M phentolamine. Incubations were for 20 min duration and were terminated by rapid filtration through Whatman GFB filters followed by two washes (6 ml each) with Tris buffer. Filters were dried out and counted in a liquid scintillation counter using an appropriate counting fluor after several hours in the dark. In the conditions described above, all three incubations were linear with protein concentrations at all ligand concentrations used here and in Fig. 1 and in the sites behaved in pharmacological displacement experiments as described in the literature^{10,11}. In particular, the ^3H -clonidine site showed the following order of displacement (at both 0.5 nM and 5 nM concentration): clonidine > adrenaline > noradrenaline > piperoxone > yohimbine > phenylephrine > WB-4104 > prazosin. This site also demonstrates modulation by GTP and analogues, some mono- and divalent cations, but not ATP (confirming the findings of refs 10, 11). Dissociation of bound ^3H -clonidine is biphasic in the absence of GTP, indicating the possible presence of multiple forms of α_2 -adrenergic receptors (see also Fig. 1 legend); in the presence of GTP (5×10^{-4} M), however, dissociation of bound clonidine was rapid, complete and monophasic. Conditions (multiple washes, large incubation volumes) were chosen to minimize these confounding variables in the study of the morphine effects; their relevance to the functioning of α_2 -adrenergic receptors in intact cells may be great but it is not the subject of this report. Additionally, in more concentrated incubations with incompletely washed fractions, they may contribute to nonlinearity in Scatchard plots or added variability in the assay. Data are presented as fmol per incubation and the relative results are the same if calculated per mg protein or per g wet weight of tissue. ^3H -Clonidine binding is about 15% higher than reported values due to the different incubation volume.

* $P < 0.05$, $n = 5$; † not significantly different from controls; ‡ $P < 0.01$ $n = 5$.

two additional days (72 h total), opiate withdrawal was tested in a few animals of each group either by the injection of naloxone (2 mg per kg) or removal of the pellet⁹. Successful treatment was indicated by the appearance of such signs as wet dog shakes, jumping, writhing and diarrhoea (1 h after naloxone or 24 h after pellet removal). The remainder of the animals in each group were killed, their brains immediately removed, dissected and frozen on dry ice.

This chronic morphine treatment (Table 1) produced an increase in ^3H -clonidine binding (α_2 -adrenergic receptors^{10,11}) to brain membranes prepared from cerebral cortex and brain stem (Table 1). As previously reported and shown here in a typical experiment (other replications are mentioned below and in Fig. 1), a smaller (but statistically significant) enhancement of 1-[^3H]dihydroalprenolol (DHA) binding (β -adrenergic receptors¹²) was also found¹³, although no significant alteration in ^3H -WB-4101 binding¹⁰⁻¹² (α_1 -adrenergic receptors) was observed. Following acute administration of morphine sulphate (50 mg per kg, intraperitoneally, 1 h previously), no significant changes in either clonidine or DHA binding were observed. Additionally, there were only small increases in clonidine binding after 1 day. The full effect takes several days to appear, consistent with the reported changes in noradrenaline turnover and cell firing²⁻⁴. Analyses of data obtained in studies of clonidine binding to membranes prepared from chronic morphine-

treated animals and controls indicated that the enhanced binding of clonidine was due to an apparent increase in the number of binding sites (Fig. 1). This was also the case (data not shown) with DHA, as demonstrated previously¹³. A single injection of morphine had no effect on either clonidine or DHA binding (Table 1), and the inclusion of up to 10^{-5} M morphine in the assay itself had no effect on control binding of either drug (data not shown).

Following morphine treatment, a single dose of naloxone will elicit severe withdrawal symptoms in the conditions described above. Additionally, within 1 h, naloxone administration entirely reversed the enhancement of both clonidine and DHA binding obtained after chronic morphine treatment (in this experiment, a $29.5 \pm 0.5\%$ enhancement of clonidine binding ($n = 5$) and a $16.5 \pm 0.3\%$ enhancement of DHA binding were obtained after morphine treatment; $P < 0.05$ in each case). Following naloxone, morphine-treated animals ($n = 5$) also treated with naloxone showed a $8 \pm 1\%$ increase in clonidine binding which was not different from that in animals treated with naloxone alone ($7 \pm 1\%$ increase compared with untreated animals ($n = 5$)). DHA binding was increased $4 \pm 1\%$ following naloxone and $7 \pm 1\%$ in morphine-treated animals also treated with naloxone. The addition of naloxone (10^{-6} M) to binding assays with tissue from either control or morphine-treated animals did not lower binding of clonidine or DHA, suggesting that naloxone had a more complex action than direct occupancy of opiate receptors leading immediately to altered clonidine binding. Interestingly, pretreatment of animals with naloxone had little effect on the binding of clonidine and DHA when compared with untreated control animals. For pharmacoc-

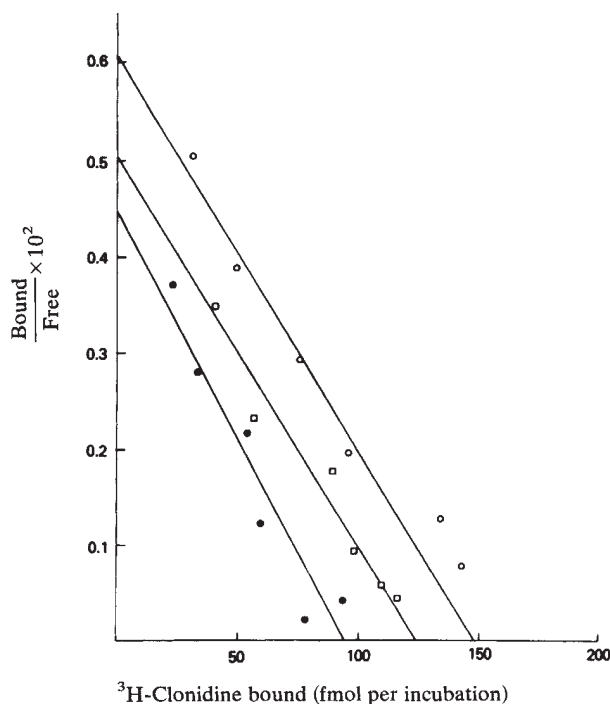


Fig. 1 Tissue was prepared as described in Table 1 legend and incubations were carried out as described except that the concentration of ^3H -clonidine was varied. Morphine treatment, either one or three pellet (over 2 days) implantation, resulted in significant increases in the apparent number of binding sites for clonidine. B_{max} values for control (●) 94 ± 8.0 , for one pellet (○) 148 ± 7.7 , and for three pellets (□) 127 ± 8.3 . No attempt was made to measure the circulating level of morphine following these implants; however, randomly selected animals from each treated group showed the signs of withdrawal described in the text. The linear Scatchard plots could be converted to broken lines by including the modifiers mentioned in Table 1 in the incubation. In the presence of GTP (10^{-4} M), resolution of ligand binding data into two components with apparent affinities of 2.1 nM and 22.5 nM was possible. Similar results were obtained with tissue from chronic-morphine treated animals.

inetic reasons, shorter times of naloxone administration to morphine-treated animals were not examined. Chronic treatment with naloxone was not attempted.

Opiate-like substances are reported to act on brain noradrenergic neurones by (1) inhibiting the firing rates of the noradrenergic neurones of the locus coeruleus, (2) reducing noradrenaline levels and/or turnover, and (3) inhibiting the depolarization-induced release of noradrenaline from cerebral slices^{7,14}. A prolonged suppression of noradrenergic activity caused by the inhibitory actions of morphine results in an increase in the apparent number of some noradrenergic target receptors, as reflected in the increased binding of ³H-clonidine. Llorens *et al.*¹³ have also reported that the binding of ³H-DHA was significantly increased (by 19%) in rat cortical membranes. β -adrenergic binding data from our study confirmed this finding.

The demonstration that there were no changes in α -adrenergic binding using the α -antagonist WB-4101, whereas specific changes were apparent using ³H-clonidine, presumably reflects occupancy of distinct populations of receptors by the two ligands. This supports the findings of other studies which report that ³H-WB-4101 binds to one class of α -receptor and ³H-clonidine to a second category demonstrating different biochemical and pharmacological characteristics^{10,11}.

The increase in binding of clonidine reported here contrasts with decreases in ³H-clonidine binding sites following chronic treatment with the monoamine oxidase inhibitor clorgyline¹⁵. Additionally, we and others have found increases following intraventricular 6-hydroxydopamine¹¹. It is interesting that both receptor changes are rapidly reversed in morphine-treated animals following abrupt withdrawal by naloxone injection, probably as a result of an increased turnover of noradrenaline¹⁶. In this regard, rapid desensitization of adrenergic receptors in brain slice experiments has been reported¹⁷ and the time course of these changes is consistent with the changes reported here. However, decreases in clonidine binding following monoamine oxidase inhibitors shows a slower time course (days), which may reflect differences in noradrenaline release following the two treatments.

The distribution of opiate and α_2 -adrenergic receptors shows some similarity in rat brain, overlapping particularly in areas implicated in opiate function^{18,19}. It is possible, therefore, that some of the signs of withdrawal (such as clonidine-reversed increased noradrenaline release¹⁶) may be related to or amplified by the development of α_2 -adrenergic supersensitivity during morphine treatment. The findings described here may be relevant to the effective use of clonidine in the treatment of withdrawal signs²⁰, and we are therefore exploring the possibility that similar receptor changes occur in humans following addiction.

Contralateral denervation causes enhanced transmitter release from frog motor nerve terminals

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It is becoming increasingly apparent that vertebrate neuromuscular junctions can exhibit considerable plasticity in transmitter release. Long-term changes in the level of release, and therefore the effectiveness of synaptic transmission, are seen in conditions of altered use^{1,2}, ageing^{3,4}, hibernation⁵, disease⁶, hormonal influence⁷ and as a result of competitive interaction⁸. We have previously shown that transmitter release at frog neuromuscular junctions can vary over a wide range depending on the type of muscle examined^{9,10} or motor unit size^{11,12}. We now present evidence that unilateral denervation of the frog sartorius muscle causes a large increase in transmitter release from nerve terminals in the contralateral sartorius, without an increase in synaptic size. Consequently, the safety margin for neuromuscular transmission is enhanced¹³. Contralateral denervation also causes a significant increase in apparent poly-neuronal innervation; but in contrast to similar experiments with the frog cutaneous pectoris muscle¹⁴⁻¹⁷, the increase in polyneuronal innervation does not seem to be due to nerve terminal sprouting.

The left sartorius muscle in 37 adult frogs (*Rana pipiens*, body length 4.5–7 cm, anaesthetized in 0.1% tricaine methane-sulphonate) was completely denervated by one of the following procedures: (1) crushing of the sartorius nerve near its entrance into the muscle; (2) sartorius nerve crush with excision of the lateral or pelvic half of the sartorius muscle; (3) resection of a 1-cm length of spinal nerve 8 near the cord. The different operative techniques, designed for other studies involving transmitter release in reinnervated ipsilateral muscles¹¹, produced essentially the same changes in contralateral muscles. Synaptic effectiveness, the incidence of polyneuronal innervation and synaptic morphology were assessed 5–16 weeks later in the intact right sartorius as described previously^{10,11} and below. Controls were normal sartorius muscles from 44 unoperated frogs, obtained from the same supplier at the same time. Muscles were immersed in Ringer solution containing 116 mM NaCl, 2 mM KCl, 1.8 mM CaCl₂, 1 mM MgCl₂ and about 1 mM NaHCO₃ (pH 7.2), or in Ringer containing reduced Ca²⁺ concentration as noted. Endplate potentials (e.p.ps) were

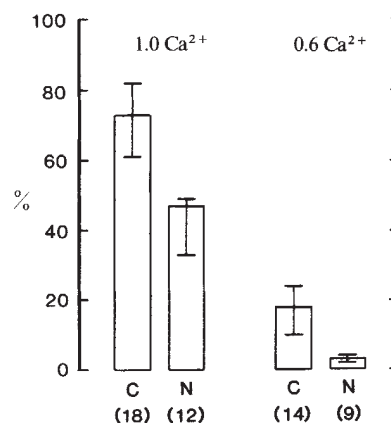


Fig. 1 The effect of lowered Ca²⁺ concentration (1.0 and 0.6 mM) on twitch tension evoked by nerve stimulation for sartorius muscles contralateral to a nerve crush (C) and normal sartorius muscles from unoperated frogs (N). Tension is expressed as a percentage of the tension generated in normal Ringer containing 1.8 mM Ca²⁺. At each concentration the median, interquartile range and number of muscles tested (in parentheses) are shown.

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recorded intracellularly with standard techniques. The direct method of estimating quantal content, that is, mean e.p.p./mean miniature e.p.p., was used. Bath temperature (15 °C) and the degree of stretch of the muscle ($1.1 \times$ rest length) were carefully controlled. Data samples showing skewed distributions are described here by their medians and interquartile ranges (i.q.r.), and were compared with the Mann-Whitney *U*-test. Parametric statistics were used for other samples as indicated.

We first assessed synaptic efficacy by measuring the tension of twitches evoked by single nerve stimuli using Ringer containing various Ca^{2+} concentrations. When the Ca^{2+} concentration is lowered, tension falls as transmission at the strongest junction on some muscle fibres becomes subthreshold. Thus, at the particular low Ca^{2+} concentrations examined (1.0 and 0.6 mM), the extent to which tension has fallen relative to the tension generated in normal Ringer (1.8 mM) is a measure of safety margins at junctions in that muscle. Because many sartorius junctions have very low safety margins and others are even subthreshold for muscle contraction in normal Ringer^{10,12}, this procedure is a sensitive test for differences in synaptic effectiveness. Figure 1 shows that when Ca^{2+} concentration was lowered to 1 mM, twitch tension in contralateral muscles fell only to 73% (median of 18 muscles) of tension in normal Ringer. This was significantly more than tension remaining in normal muscles (47%, 12 muscles, $P < 0.002$). Similarly, in 0.6 mM Ca^{2+} residual twitch tension in contralateral muscles (18%, median of 14 muscles) was significantly higher than in normal muscles (3%, 9 muscles, $P < 0.002$). Similarly enhanced safety margins were seen at the longest post-operative time studied (111 days, 24% in 0.6 mM Ca^{2+}).

The second method of testing synaptic effectiveness was to

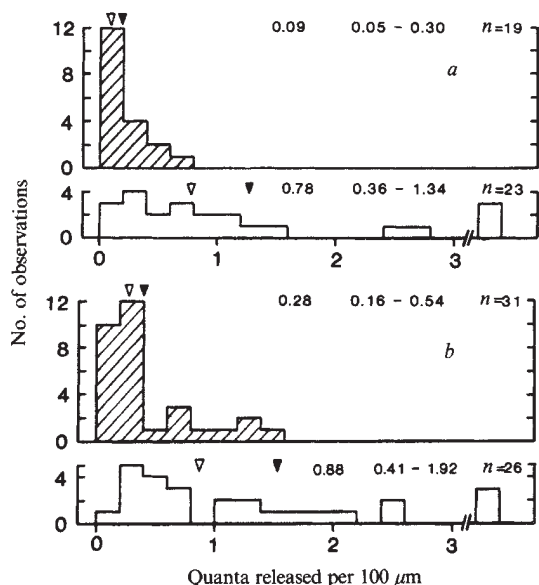


Fig. 2 Quanta released per 100 μm of nerve terminal length in Ringer containing 0.2 (a) or 0.3 mM Ca^{2+} (b) and 1 mM Mg^{2+} . In each case the upper (shaded) histogram shows data from junctions in normal unoperated sartorius muscles and the lower (unshaded) histogram data from muscles contralateral to a denervation. Figures shown are median (also indicated by ∇), interquartile range and sample size. Means are indicated by $\blacktriangledown$. Actual data exceeding 3 quanta per 100 μm are 3.21, 4.49 and 5.13 in a and 4.84, 5.39 and 7.41 in b. Normalizing release to terminal length presupposes that release and terminal length are directly proportional. Such proportionality seems to hold for frog muscle when release is partially blocked by low Ca^{2+} concentration¹⁹, but the correlation is marginal. In our experiments there was a significant positive correlation between quantal content and terminal length for normal sartorius junctions, but not for contralateral junctions. A non-correlation might be expected if release is highly non-uniform along the length of a terminal branch at low Ca^{2+} concentrations²⁵ (but see ref. 26). Such non-uniformity would affect consideration of possible physiological mechanisms of enhancement, but not the basic observation of greater release.

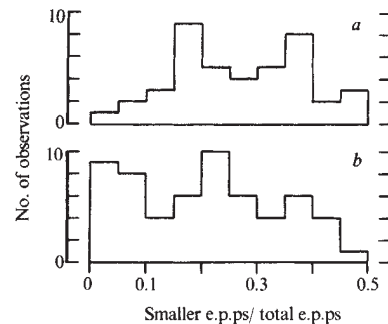


Fig. 3 For doubly innervated endplate sites, these histograms show the distributions of the ratios of the amplitude of the smaller e.p.p. component to total e.p.p. amplitude. a, Normal sartorius muscles, $n = 42$, mean ratio = 0.28; b, sartorius muscles contralateral to a denervation, $n = 58$, mean ratio = 0.22. Curare concentration $2-5 \times 10^{-6}$ M.

measure transmitter release in terms of e.p.p. quantal content in Ringer containing 0.2 or 0.3 mM Ca^{2+} and 1 mM Mg^{2+} . In 0.2 mM Ca^{2+} , quantal content at junctions in contralateral muscles (median = 3.38, i.q.r. = 1.60–8.01, $n = 23$) was fivefold greater than at junctions in normal muscles (0.67, 0.21–1.75, $n = 21$), and in 0.3 mM Ca^{2+} , quantal content at contralateral junctions (median = 4.80, i.q.r. = 2.05–9.22, $n = 28$) was nearly threefold higher than at normal junctions (1.65, 0.97–2.87, $n = 45$). These differences were highly significant (0.2 mM Ca^{2+} : $P < 0.001$, 0.3 mM Ca^{2+} : $P < 0.001$).

To determine whether this increase in transmitter release was due to an increase in synaptic size, contralateral and normal muscles were stained with the Karnovsky cholinesterase (ChE) stain¹⁸. As endplate size is proportional to muscle fibre diameter¹⁹ we made sure that the muscle fibres bearing the endplates in the samples we compared had similar distributions of diameter (mean contralateral fibre diameter = 47 μm , mean normal fibre diameter = 46 μm). Endplate size, measured as total ChE-stained postsynaptic gutter length, was not significantly different for contralateral junctions (402 ± 11 μm s.e.m.; 333 endplates from 19 muscles) and normal junctions (416 ± 15 μm s.e.m.; 190 endplates from 8 muscles; $P > 0.40$). Thus, the enhanced quantal content at contralateral junctions apparently cannot be explained by an increase in synaptic size; but rather the inherent release properties of contralateral terminals seem to have been altered.

This conclusion is supported by measurements of the efficacy of the release mechanism in identified terminals. Junctions from which recordings were made were marked by intracellular dye injection, stained with nerve terminal (see below) and ChE stains, and measured in the light microscope. Quantal contents were then normalized to nerve terminal length and the result, expressed as quanta released per 100 μm of nerve terminal, can be viewed as a measure of the inherent transmitter release capability of a given terminal. Figure 2a shows that in 0.2 mM Ca^{2+} , median release per unit length was over eight times higher in contralateral muscles than in normal muscles (0.78 compared with 0.09), whereas in 0.3 mM Ca^{2+} (Fig. 2b) it was over three times higher (0.88 compared with 0.28). These differences were highly significant (0.2 mM Ca^{2+} : $P < 0.001$, 0.3 mM Ca^{2+} : $P < 0.005$). Mean values are also shown in Fig. 2 (closed arrows), but in such skewed distributions they describe the average less reliably. We found no evidence for any seasonal variation in any of our measurements of synaptic effectiveness.

In other intracellular measurements, we sought to estimate the incidence of focal polyneuronal innervation, defined as the convergence of two or more motor axons on to the same endplate site. Sartorius muscle fibres also show nonfocal polyneuronal innervation, that is, two or more well-separated endplate sites occur along the length of each fibre. Muscles were immersed in Ringer containing the minimum concentration of curare ($2-5 \times 10^{-6}$ M) necessary to block twitching. We then tried to subdivide the e.p.p. by changing the amplitude, polarity

and duration of a stimulus to the nerve. This procedure, although customary, almost certainly underestimates polyneuronal innervation. In addition, we restricted our comparison of polyneuronal innervation to muscles tested at the same time of year, because there is evidence that the occurrence of terminal sprouting in normal frogs shows seasonal variation²⁰.

We found that the incidence of apparent polyneuronal innervation was higher in sartorius muscles contralateral to a denervation (15% = 59/392 endplates from 11 muscles) than in normal sartorius muscles (9% = 21/227 endplates from 6 muscles) when both types of muscles were tested at the same time of year (May–June). The difference between these proportions was significant ($P < 0.02$).

A search was made for morphological correlates of the higher incidence of focal polyneuronal innervation in contralateral muscles relative to normal muscles. Muscles were stained with the nitroblue tetrazolium (NBT) nerve terminal stain²¹ and with ChE stain. NBT renders all nerve terminals and terminal sprouts clearly visible in the light microscope (Zeiss 40/0.75 water immersion objective, final magnification $\times 320$). For example, the fine terminal sprouts which appeared commonly in the reinnervated sartorius muscles used in other studies¹¹ were consistently stained by the identical procedure. Careful examination of 30–50 well-exposed surface endplates in each of 10 contralateral muscles revealed no nerve terminal sprouts between synaptic sites on adjacent muscle fibres. In all other respects, contralateral junctions were indistinguishable from normal junctions in the light microscope. Thus, in contrast to reports for cutaneous pectoris muscles contralateral to a denervation^{14,17}, we find no morphological evidence in contralateral sartorius muscles either for nerve terminal elongation or terminal sprouting to innervate adjacent fibres. We cannot rule out the possibility that collateral (nodal) sprouts, or preterminal sprouts (branches from the unmyelinated portion of an axon proximal to the terminal) are responsible for the increased polyneuronal innervation. However, we see no terminal sprouts of the type described by Rotshenker and Reichert^{14,17} in contralateral cutaneous pectoris muscles. In the absence of such evidence, an alternative explanation for the apparent increase in focal polyneuronal innervation can be suggested.

Our estimate of focal polyneuronal innervation in the normal sartorius is in the range of previous estimates of 6–30% (refs 22–24). Recently, however, we found that sartorius endplates may receive some inputs which are so weak that they may be easily obscured by the curare used to assess polyneuronal innervation¹². If contralateral denervation causes enhanced release, some of the very weak polyneuronal inputs may become sufficiently strong to be detectable in curare. Figure 3 shows the distribution of the ratios of the amplitude of the smaller e.p.p. component relative to total e.p.p. amplitude at doubly innervated endplates sites in normal muscles (Fig. 3a) and contralateral muscles (Fig. 3b). The two histograms differ in that a greater proportion of the contralateral doubly innervated endplates receive one input which is much weaker than the other. Of the 58 doubly innervated contralateral endplates 17 (29%) show a ratio of e.p.p. components ≤ 0.1 , whereas only doubly innervated normal endplates (7%) fall into this class. In the absence of any morphological evidence for terminal sprouting, it seems possible that the apparent increase in polyneuronal innervation at our contralateral endplates is due to the enhanced detectability of normally present polyneuronal inputs.

Further studies emphasized that for comparative purposes, it was important to control for seasonal variation in polyneuronal innervation. Normal sartorius muscles tested in mid-winter (January–February) showed enhanced polyneuronal innervation (18%, 56/311 endplates from 7 muscles). We are pursuing this seasonal difference.

Many questions remain unanswered regarding this contralateral effect. It is not clear, for example, whether the increase in transmitter release predominantly affects weak terminals as may be inferred from Fig. 3, or whether all terminals are involved. Further, our findings differ from those of Weakly and Yao²⁴,

who found no change in polyneuronal innervation of frog sartorius junctions after contralateral denervation. We have no specific explanation for this discrepancy, except to suggest that polyneuronal innervation, as well as synaptic effectiveness and sprouting, are probably controlled in complex ways by several factors. We are now investigating the physiological mechanisms of the enhanced transmitter release, related effects in contralateral and ipsilateral synergists and antagonists, and the involvement of the spinal cord as a possible pathway for the effect. The results of these studies may have important implications for the interpretation of other experiments, using spinal isolation, deafferentation, tenotomy and local paralysis. It is clear, for example, that contralateral muscles may not serve as adequate controls for unilateral manipulations.

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Ca-dependent K channels with large unitary conductance in chromaffin cell membranes

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Injection of Ca ions increases the membrane permeability of many types of cells (reviewed by Meech¹), and in molluscan neurones, removal of Ca ions from the external solution suppresses a voltage-dependent K conductance^{2,3}. These two phenomena may be related through a potential-dependent Ca influx². The question then arises whether the voltage dependence of the corresponding K permeability system is an intrinsic property of this system or whether it is merely a consequence of the increase of Ca influx with depolarization. The resolution of this and similar problems has been hampered by technical difficulties, due to the presence of voltage-dependent Ca channels, of Ca-independent K conductances and of strong intracellular buffering for Ca ions. In the present study patches of membranes containing functional, Ca-dependent K channels have been isolated from chromaffin cells. It is shown that application of low Ca concentrations to the inner side of the membrane affects the properties of the channels, while Ca ions are ineffective on the outer side of the membrane. The method used here overcomes several of the limitations of the previous studies using cellular recording.

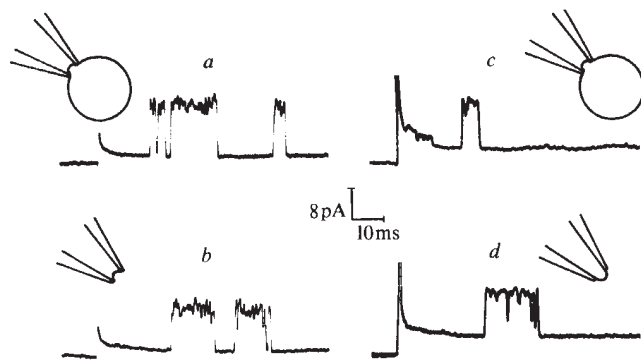


Fig. 1 Large K unitary currents in isolated patches. *a, b*, A pipette containing Ca-free Ringer (1 mM Co replacing Ca) was first used to record from a patch of a cell membrane (*a*). The bath contained a high-K solution (in mM: 143 KCl, 2 MgCl₂, 1 Ca-11 EGTA buffer, 10 HEPES-KOH, pH 7.3) which presumably brought the cell membrane potential close to 0 mV. The pipette was held at the bath potential, so that the holding potential for the patch was also 0 mV. *a*, 75-mV potential jumps were applied to the pipette interior to make the patch membrane potential positive. *b*, Response to the same voltage pulse after moving the pipette away from the cell (inside-out patch). Unitary outward currents are 12 pA in *a*, 10.5 pA in *b*. There is a pronounced noise increase during the opening of the channels. *c, d*, A pipette containing a high-K solution (same as the bath solution in *a, b*) was first applied to a cell bathed in normal Ringer (*c*). The pipette interior was held at the bath potential so that the patch holding potential was equal to the cell resting potential (presumably ~ -60 mV). 100-mV positive pulses activate large (11 pA) K unitary currents. *d*, Similar currents are obtained after destruction of the initial patch and withdrawal of the pipette when applying 104-mV pulses from a holding potential of -64 mV (outside-out patch). 1 kHz filtering.

Isolated bovine chromaffin cells⁴ were cultured for up to 8 days in medium 199 (Gibco) supplemented with 10% fetal calf serum, bovine serum albumin (1 mg ml⁻¹) and antibiotics. They remained in good condition as judged by their ability to grow dendrite-like structures, to maintain a high resting potential and to fire action potentials. High-resolution measurements of currents were obtained through a glass micropipette tightly sealed onto a patch of membrane ('gigaseal' method⁵⁻⁸). The potential in the pipette was under experimental control, while the potential on the other side of the membrane was either the cell resting potential or, in isolated patches, the bath potential. In all experiments, a large-resistance (>10 G Ω) seal was established between the pipette and the membrane of the intact cell. Large unitary outward currents were then obtained on depolarizing the patch membrane (Fig. 1*a, c*). The corresponding channels will be fully characterized elsewhere; they are K selective (as indicated by a reversal potential value more negative than -60 mV in normal Ringer and of about 0 mV in isotonic K solution) and have a very high unit conductance (~ 180 pS in symmetrical K). The frequency of the current pulses at a given potential is strongly reduced if Ca ions are omitted from the pipette solution. Compared with the Ca-dependent single K-channel recordings recently described in *Helix* neurones⁹, they differ by having a higher unit conductance and by the lack of a delay in turn-on on large depolarizing voltage commands.

After forming a seal, cell-free patches of membrane were isolated in two ways. In one type of experiment, the pipette was simply moved away from the cell in a low-Ca, high-K medium to yield an 'inside-out patch'⁶⁻⁸ (Fig. 1*a, b*). Positive potential jumps from 0 mV activated large outward unitary currents with very similar properties before and after withdrawal of the pipette from the cell. (Voltage differences are given with respect to the patch membrane: the potential on the outer side is taken as zero. Current directions are also referred to the patch, with outward membrane currents being displayed upwards in the records.) In the other type of experiment, the initial patch was destroyed by applying suction to the pipette interior and the

pipette was then withdrawn. In most cases it then sealed over with a new patch of membrane pulled away from the cell⁸ ('outside-out patch', Fig. 1*d*). Whereas inside-out patches have the internal (previously intracellular) side of the membrane in contact with the bath, outside-out patches have their external side exposed to the bath⁸.

Initial attempts to change the bath solution with various Ca-EGTA buffers in the conditions of Fig. 1*b, d* led to erratic results, probably because vesicular structures remained at the pipette tip instead of the desired single membrane patches⁵. These vesicular structures were avoided in later experiments. In the inside-out configuration (Fig. 1*b*), the outer part of the vesicle was destroyed chemically (Ca-EGTA buffer) and, if necessary, mechanically (by exposing briefly the pipette tip to air, or by bringing it in contact with a hexadecane droplet formed at the tip of a capillary glass tube held in the bath). None of these methods has a 100% success rate; the experiment was not continued until there was good evidence that a genuine inside-out patch had been obtained. (In cell-free conditions, the channels should have a unit size and kinetic properties virtually identical to those observed before leaving the cell, as illustrated in Fig. 1*a, b*.) In the outside-out configuration (Fig. 1*d*), the time constant of the whole-cell membrane capacitive current was measured when the initial seal had been destroyed by suction and the pipette was still in contact with the cell. Low values (<0.2 ms) of this time constant ensured a low (<20 M Ω) resistance through the initial patch which could then be considered as effectively destroyed. With these precautions, the exchange of the bath solution (in the conditions of Fig. 1*b, d*) gave fast, stable and reliable results on the channel properties. The bath contained about 1 ml of solution, and was continuously perfused at a rate of ~ 1 ml min⁻¹. Normal Ringer contained (in mM): 140 NaCl, 2.8 KCl, 2 MgCl₂, 1 CaCl₂, 10 HEPES-NaOH, pH 7.2-7.3. The solutions used on the inner side of the membrane contained 143 KCl, 2 MgCl₂, 10 HEPES-KOH, pH 7.2-7.3, and a Ca-EGTA buffer. The effective free Ca concentration, Ca_{in}, was calculated using an apparent dissociation constant of 10^{-7} M (estimated from ref. 10). All experiments were carried out at room temperature.

If the Ca concentration is altered at the external side of an outside-out isolated patch (Fig. 1*d* situation), no conspicuous effect is seen on the K channels. An example of such an experiment is illustrated in Fig. 2.

In sharp contrast, Fig. 3 shows the striking effects that follow a change in Ca-EGTA buffers on the internal side of an inside-out

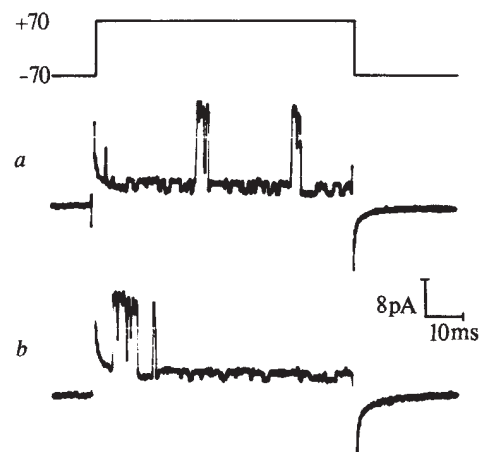


Fig. 2 Lack of effect of external Ca in an outside-out patch. 140-mV positive pulses were applied from -70 mV in an outside-out patch bathed in normal Ringer (*a*) and in 0 Ca, 1 mM Co Ringer (*b*). The pipette contained a high-K solution and a Ca-EGTA buffer with a calculated Ca concentration of 2×10^{-7} M. The voltage jumps elicit large (15 pA) K currents as well as smaller outward currents (see also Fig. 1*c*) not studied here. Suppressing the external Ca ions does not affect the large K currents. 2 kHz filtering.

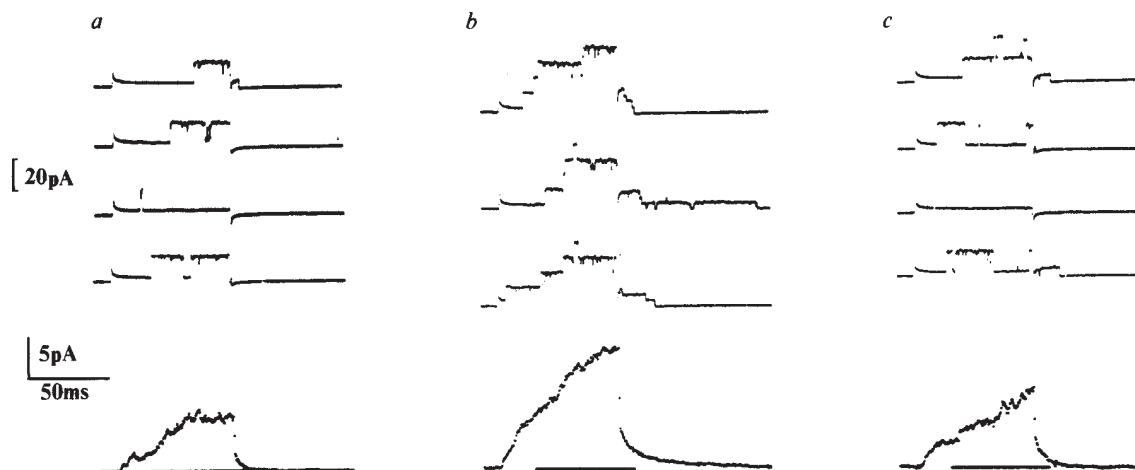


Fig. 3 Effects of various Ca-EGTA buffers in an inside-out patch. An inside-out patch was exposed to a high-K solution containing 1 mM Ca + 11 mM EGTA (*a*, *c*) and 1 mM Ca + 1.5 mM EGTA (*b*). Calculated free Ca concentrations are 10^{-8} M in *a* and *c*, and 2×10^{-7} M in *b*. 67-mV positive potential jumps from a holding potential of 0 mV were applied at ~ 1 Hz. The pipette contained Ca-free Ringer (1 mM Co replacing Ca). *a*, Four records in the initial low-Ca solution (upper traces). The unitary current is 4.5 pA at 0 mV, and 13.4 pA at +67 mV. Lower trace: average of 50 consecutive sweeps (the background current has been subtracted). *b*, Higher Ca solution. The unitary current is 4.0 pA at 0 mV and 9.6 pA at +67 mV. The number of open channels is larger during the pulse than in *a* and there is a marked lengthening of the tail of activity after returning to 0 mV. These two features are also evident in the average of 47 consecutive sweeps (lower trace). *c*, Records taken ~ 1 min after returning to the initial solution. The unitary current is 5.0 pA at 0 mV and 13.2 pA at +67 mV. The kinetic properties of the currents are almost as in *a* (lower trace, 37 consecutive sweeps). 1 kHz filtering. Vertical scale: 20 pA for single sweeps (upper traces) and 5 pA for average currents (lower traces). Baseline is indicated by horizontal bars in average current traces.

patch (Fig. 1*b* situation). The buffers used had estimated free Ca concentrations of 10^{-8} M and 2×10^{-7} M. Positive potential steps of 67 mV were applied from 0 mV. The elementary current is lower at the higher Ca concentration, with ratios of 0.89 at 0 mV and 0.72 at 67 mV. The average number of open channels is 0.51 at the end of the positive pulse and $\sim 5 \times 10^{-4}$ before the pulse in 10^{-8} M Ca. This contrasts with 1.7 and 0.02 respectively in 2×10^{-7} M Ca. These values show that Ca increases the average number of open channels. All effects were readily reversible (Fig. 3*c*).

Figure 3 typifies results obtained with Ca-EGTA buffers having calculated Ca_i values of 10^{-9} M, 10^{-8} M (Fig. 3*a*), 5×10^{-8} M, and 2×10^{-7} M (Fig. 3*b*). All Ca effects illustrated in Fig. 3 were gradually observed on this concentration scale. In control experiments, the effects of two Ca-EGTA buffers having

the same calculated Ca_i value but different EGTA concentrations were compared. These buffers (in mM: 11 EGTA-1 Ca and 1.1 EGTA-0.1 Ca) had identical effects on the K channels indicating that EGTA *per se* is ineffective. This result contrasts with a previous report on perfused snail neurones¹¹.

Figure 4 illustrates the result of changing the bath solution to one containing 1 mM Ca (and no EGTA). The unitary conductance is decreased, the mean number of open channels greatly increased and the voltage sensitivity entirely lost. The channels have open times of ≥ 1 s (not illustrated). Again, all effects are reversible. Furthermore, application of 1 mM Ca_i to inside-out patches at membrane potentials of $\geq +60$ mV resulted in multiple interruptions (of ~ 1 ms each) of the single channel currents, like those observed with QX222 on acetylcholine-sensitive channels¹². These interruptions are probably due to a

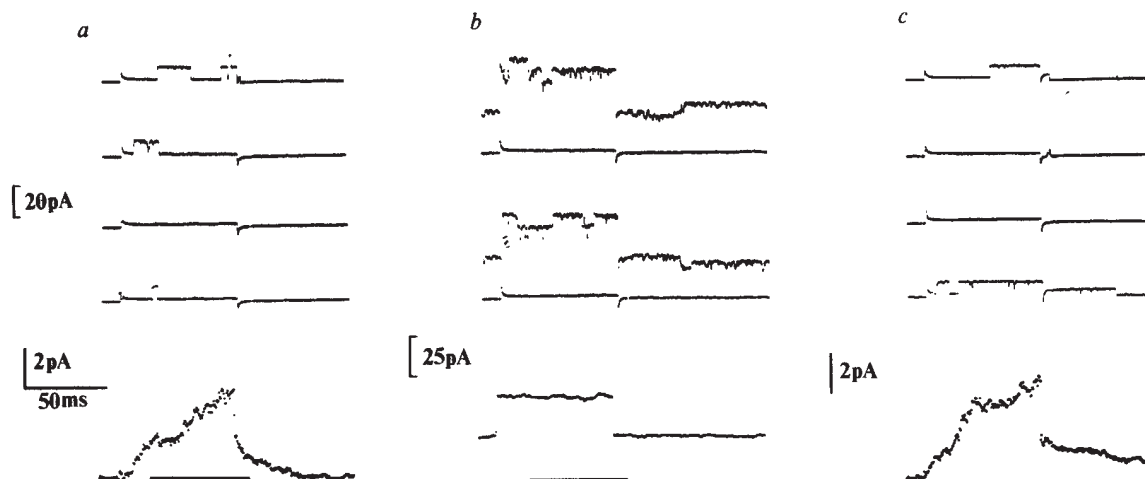


Fig. 4 Effects of 1 mM Ca in an inside-out patch. 40-mV positive potential jumps were applied from a holding potential of 0 mV to the same patch as in Fig. 3. *a*, Same buffer as in Fig. 3*a*. *b*, The bath was changed to a 1 mM Ca-0 mM EGTA solution. *c*, After returning to the initial buffer (recordings start 30 s after the solution change). Single sweeps (upper traces) and averaged currents (lower traces; 51, 21 and 48 consecutive sweeps in *a*, *b* and *c*, respectively) are illustrated. The background currents in the two upper traces in *b* were taken shortly after returning to the EGTA buffer. The unitary currents are at 0 mV, 4.2 pA (*a*), 3.8 pA (*b*) and 4.0 pA (*c*); at +40 mV, they are 8.0 pA, 7.2 pA and 7.6 pA, respectively. Statistical analysis of the traces in *b* suggests that 10 active channels were present in the patch. This value makes the probability of channel opening to be 0.76 in the presence of 1 mM Ca. (The probability of opening in the other traces of Figs 3 and 4 can be similarly calculated as the mean number of open channels divided by 10.) 1 kHz filtering. Vertical scale: 20 pA for single sweeps; 2 pA (*a*, *c*) and 25 pA (*b*) for average currents. Baseline indicated by horizontal bars in average current traces.

blockade of the channels by internal Ca ions. Previous observations of a reduction of K currents after injection of Ca ions into molluscan neurones³ may be, at least in part, related to such an effect.

The present work is the first report of Ca-dependent K channels in chromaffin cells. These channels display an unusually large unitary conductance, and their properties are affected by the Ca concentration on the inner side of the membrane. In intact cells this concentration may be locally altered by voltage-dependent inflow of Ca ions as suggested by Meech and Standen². Clearly, the action of Ca does not require any other soluble cytoplasmic substance.

For low internal calcium concentrations, the Ca-dependent K channels of chromaffin cells are voltage dependent. However, their sensitivity to the membrane potential is affected by Ca, and at 1 mM inner Ca concentration no voltage dependence is observed. Some investigators^{13,14} have found that the K conductance elicited by injections of Ca ions to molluscan neurones is virtually voltage independent, whereas others have found a voltage-dependent conductance^{15,16}. The present work suggests that the former results were simply obtained with larger concentrations than the latter.

Finally, the results show that the unitary conductance of K channels depends on Ca_i. This variation is already observable for very low Ca concentrations. Probably some common mechanism underlies this phenomenon as well as the effects of Ca_i on the channel opening probability and its voltage dependence.

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Monoclonal antibodies against human fibroblast interferon

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The advantages of using monoclonal antibodies account for current interest in antibodies specific for interferon^{1–3}. The successful isolation of a hybridoma line secreting mouse monoclonal antibodies to human leukocyte interferon (IFN- α) has already been reported⁴. We have now established a stable hybrid myeloma line (FO) which secretes antibody against human fibroblast interferon (IFN- β). We have demonstrated that, even after removal of much of the sugar moiety of IFN- β , the monoclonal anti-IFN- β antibodies neutralize the antiviral activity of human IFN- β but not that of human IFN- α . We suggest the possible use of these monoclonal antibodies for the identification and purification of IFN- β expressed by DNA cloned in bacteria.

Table 1 Inhibitory effect of anti-IFN- β antibody preparations on IFN- β antiviral activity

Anti-IFN- β antibody preparation	50% inhibition of IFN- β antiviral activity
Culture supernatant	1:7 dilution
Ascites	1:70 dilution

Various dilutions of monoclonal antibody were tested against IFN- β in the direct antiviral interferon assay on human FS4 fibroblasts with vesicular stomatitis virus (1,000 plaque forming units per ml) as challenge. The antibody titrations were carried out using 40 μ l of diluted antibody per well of the microtitre plate. Three units of IFN- β were used for each antibody sample. Ascites tumours were obtained from mice pretreated with Freund's adjuvants injected with 10⁶ hybridoma cells into the peritoneal cavity. After 2–3 weeks ascites fluids were collected and cells removed by centrifugation. Crude IFN- β was purified using an aqueous two phase polyethyleneglycol phosphate system¹⁰. Human IFN- β enters the upper phase while the bulk of the protein is found in the lower phase. The yield was 74–94%, corresponding to 3–7.3 $\times$ 10⁶ units per mg protein.

One μ g of purified blue Sepharose adsorbed IFN- β (ref. 5) was injected each week into 8-week-old female BALB/c mice; those having an anti-IFN antibody titre that inhibited human IFN- β in a direct antiviral assay⁶ at a serum dilution of 1:200 were chosen for lymphocyte fusion experiments. A final booster injection of 1 μ g IFN- β with the sugar moiety removed by treatment with a mixture of sugar-degrading enzymes was administered 4 days before spleen excision.

Myeloma FO⁷ and spleen cells were fused using the method of Köhler and Milstein¹ with the following modifications. First, a feeder layer was omitted; instead the hybrids were grown in HAT (hypoxanthine-aminopterin-thymidine) medium containing 1% methylcellulose. Individually growing hybridoma colonies were picked up with sterile capillaries after 2 weeks and cloned separately in Costar tissue plate wells. The hybridoma colonies were then tested for inhibition of antiviral activity of IFN- β on FS4 fibroblasts in an indirect antiviral assay⁴. Positive cultures were subcloned, grown for 4 days and tested using an enzyme linked immunosorbent assay according to Towbin *et al.*⁸ and a direct antiviral IFN- β assay. Cultures which were positive for antibody secretion in both the enzyme linked immunosorbent assay (ELISA) and the antiviral assay were subcloned once more and the supernatant of these again tested in the direct antiviral IFN- β assay⁶. In this way a stable hybridoma line secreting monoclonal IFN- β antibodies was established and checked for constant antibody secretion over a period of several months.

Inhibition of the antiviral activity of human IFN- β with monoclonal antibodies from hybridoma Costar tissue plate cultures was tested using increasing dilutions of the antibody sample. Partial inhibition of the antiviral activity of IFN- β was observed with antibody diluted by as much as 1:20. After treatment of the antibody sample with trypsin (10 μ g ml⁻¹) for 60 min at 37 °C and subsequent inhibition of trypsin with soybean trypsin inhibitor, the inhibitory effect of the antibody sample on IFN- β in the direct antiviral assay was completely lost, which proves that the inhibition of the antiviral interferon activity was due to a protein. In addition, to determine whether the monoclonal antibodies were directed against the sugar or the protein moiety, human IFN- β was treated with a mixture of sugar-degrading enzymes (neuramidase, α -glucosidase, α -mannosidase, α/β -galactosidases) and this interferon sample used as standard IFN- β in the direct antiviral assay. The antibodies seem to be directed against the protein part of IFN- β as the antiviral activity of the treated sample was still completely inhibited by the antibodies at the same dilution. It was shown that monoclonal anti-IFN- β antibodies do not cross-react with human IFN- α (from 'buffy coat'). Aliquots of immunoglobulin-secreting hybridoma cells were injected into the peritoneal cavity of female BALB/c mice which had been pretreated with Freund's adjuvants 1 week before to grow ascites. After 1 week, ascites fluid was collected from the peritoneal cavity containing

the monoclonal antibodies. This sample was found to have a 10-fold higher titre against standard human IFN- β in a direct antiviral interferon assay when compared with supernatant of the original monoclonal cultures (Table 1).

In an additional assay partially purified IFN- β was subjected to SDS-gel electrophoresis using the method of Laemmli⁹ in parallel with molecular weight protein standards and then electrophoretically blotted on to a nitrocellulose sheet by a method described elsewhere⁸. While the standard proteins were stained with Coomassie brilliant blue the blotted interferon sample was assayed in an ELISA test developed for protein blots, using monoclonal anti-IFN- β antibodies (Fig. 1). Figure 1 shows that the antibodies specifically react with the protein band possessing a molecular weight (MW) of $\sim 19,000$. Monoclonal anti-IFN- β antibodies which were initially purified by 35% ammonium sulphate precipitation followed by passage over a Sephadex G-200 column were coupled to 2 ml of cyanogen bromide-activated Sepharose (Pharmacia) and tested for specific immunoabsorption of IFN- β . Figure 2 demonstrates that the antibodies were indeed specific for IFN- β .

In an ELISA microtitre assay (not shown), anti-IFN- β monoclonal antibodies did not cross-react with a total protein

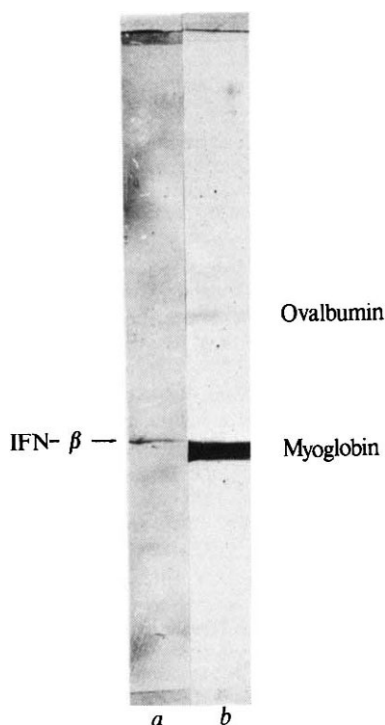


Fig. 1 Electrophoretic transfer of IFN- β from an SDS gel onto nitrocellulose sheet and subsequent ELISA reaction. *a*, IFN- β (ELISA); *b*, molecular weight standards stained with amino black. Electrophoresis was performed according to Laemmli⁹. The gel was then soaked in buffer containing 50 mM NaCl, 2 mM Na-EDTA, 4 M urea, 0.1 mM dithiothreitol, 10 mM Tris-HCl, pH 7.0, according to Bowen *et al.*¹² and the proteins (IFN- β and markers) were electrophoretically transferred onto a nitrocellulose sheet⁸. While the section of the blot containing the markers was stained with amido black, the part containing IFN- β was soaked for 4 h at 37 °C in 3% bovine serum albumin (BSA)/Tris-buffered saline to saturate all nonspecific protein-binding sites for ELISA. After washing, the monoclonal antibody was added in a 1:500 dilution in 5% horse serum/0.5% BSA for 16 h at room temperature. The blot was then soaked for 2 h in a rabbit antimouse peroxidase-conjugated immunoglobulin G (IgG) solution (1:500 dilution in 5% inactivated horse serum/0.5% BSA), washed again then soaked for a further 2 h in a goat anti-rabbit peroxidase-conjugated IgG solution (1:500 dilution as above) to make the ELISA reaction more sensitive. After a final wash, the colour reaction was performed with *O*-dianisidine as substrate⁶. Marker proteins were ovalbumin, 45,000 (45K) and myoglobin, MW 17.5 K.

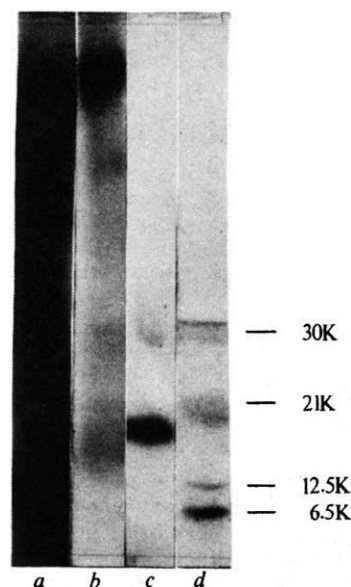


Fig. 2 Autoradiograph of an SDS gel with *a*, unpurified ¹²⁵I-labelled IFN- β (4-day exposure); *b*, unpurified ¹²⁵I-labelled IFN- β (1-day exposure); *c*, purified ¹²⁵I-labelled IFN- β (4-day exposure); *d*, protein markers. Monoclonal antibodies against IFN- β were purified from ascites fluid by a 35% ammonium sulphate precipitation followed by separation over a Sephadex G-200 column in a Tris-glycine buffer, pH 7.6, containing 0.04% *n*-butanol. The purified antibodies were then coupled to cyanogen bromide-activated Sepharose 4-B. An IFN- β sample, iodinated with ¹²⁵I according to Hunter and Greenwood³, was purified over the column. A neutral wash with Tris-buffered saline at pH 7.4 removed nonspecifically adsorbed protein material. Specifically bound ¹²⁵I-labelled IFN- β was then washed off the column with 0.1 M glycine-HCl buffer, pH 2.3. The eluted sample was lyophilized, and then electrophoresed on an SDS gel according to Laemmli⁹ parallel with unpurified ¹²⁵I-labelled IFN- β and ¹⁴C-labelled protein markers. After electrophoresis the gel was soaked twice for 10 min in dimethyl sulphoxide (DMSO), and then in DMSO/30% diphenyloxazole for 20 min, washed with H₂O and dried. The dried gel was autoradiographed.

preparation of *Escherichia coli*. Thus, as the monoclonal antibodies are probably directed specifically against the protein part of IFN- β , they could perhaps be used for the identification and purification of IFN- β expressed by DNA cloned in bacteria.

It is possible that our two stable hybridoma lines which secrete monoclonal anti-IFN- β antibodies are directed against different determinants of the IFN- β protein moiety. Note that there were fluctuations in the inhibitory activity of the monoclonal antibodies, between antiviral interferon assays which may be either due to the relative insensitivity of the assay system itself or the existence of more than one human IFN- β gene^{10,11}.

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Production of both macrophage activating and inhibiting activities by a murine T-lymphocyte hybridoma

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T lymphocytes are known to secrete a variety of factors which affect macrophage function¹, including those which inhibit migration (MIF) and activate macrophages (MAF) to become cytotoxic¹, and produce neutral proteases², complement components³ and other factors. Physical separation of MIF from MAF has not been achieved⁴ and efforts directed towards characterizing these factors have been limited chiefly by their generation from lymph nodes or spleen cells and the difficulty in performing most macrophage function assays. We elected to approach the problem of generation of lymphokine by fusing concanavalin A (Con A)-activated AKR spleen cells to the AKR T-cell lymphoma BW5147 and measuring the biological activity using established assays. We describe here the generation of a cloned T-cell hybridoma whose supernatants produce both inhibition of macrophage migration and activation of macrophages for the production of C2 and elastase.

The hypoxanthine/aminopterin/thymidine (HAT) sensitive variant of BW5147 was fused using polyethylene glycol (Sigma, molecular weight 1,000) to Con A-activated AKR spleen cells⁵. Resultant hybridomas were divided into 51 2-ml aliquots and grown in the presence of HAT-selective medium. Thirty days post-fusion only five colonies exhibited macroscopic growth. The supernatants from these colonies were screened first for inhibition of macrophage migration (Table 1); control supernatants were collected from BW5147 growing in the same medium but lacking aminopterin at 5×10^5 cells ml⁻¹. The supernatants from two colonies significantly inhibited macrophage migration in the agarose droplet microassay but three did

Table 1 Inhibition of macrophage migration and enhancement of macrophage C2 production induced by T-hybridoma supernatants

	Colonies		No.	Clones of W38	
	Migration index	Units per ml		Migration index	Units ml ⁻¹
		C2			C2
W38	0.55	0.24	1	0.62	0.78
W25	0.68	0.38	2	0.97	0.31
W31	1.08	0.12	3	0.85	0.35
W8	1.36	0.11	4	0.87	0.48
W32	1.29	0.145			
BW5147		0.095			
AKR spleen cell medium					0.12
AKR spleen cell pulsed with $6 \mu\text{g ml}^{-1}$ Con A				0.74	0.28

Inhibition of migration of C57BL/6 oil-induced macrophages, as measured by the agarose droplet technique⁷. Migration was performed in a 1:1 (colonies) or 1:4 (clones) dilution of test medium in RPMI 1640 and migration areas were measured at 20 h with a Zeiss inverted microscope. Results are expressed as the migration index, using the migration area given with BW5147 supernatants as the denominator. Final test volume per well was 0.2 ml. Production of C2 by oil-induced C57BL/6 macrophage monolayers, stimulated with supernatants from T-cell hybridomas. Macrophages at a concentration of 10^7 ml⁻¹ in RPMI 1640 containing 10% fetal calf serum were allowed to adhere overnight to Costar 2-ml 'cluster wells' and then washed free of non-adherent cells. A 1:10 dilution of test medium in RPMI 1640 was added and aliquots were removed after 60 h of incubation and assayed for C2 by the haemolytic technique of Borsos *et al.*⁸. Final test volume per well was 2 ml.

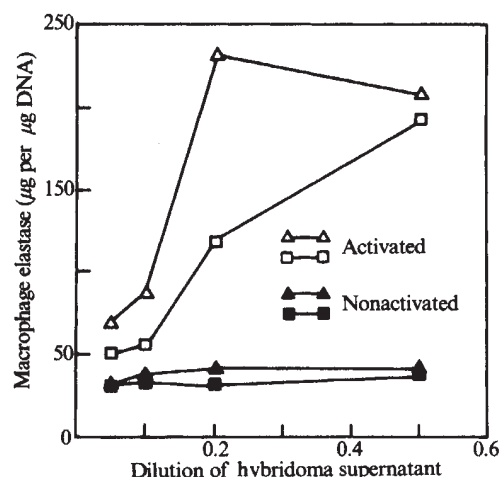


Fig. 1 Elastase production by macrophages activated with T-hybridoma W38C1 supernatants (activated, open symbols; nonactivated, solid symbols). Oil-induced C57BL/6 macrophages were prepared as for C2 production but after attachment and an overnight incubation with serial dilutions of hybridoma supernatant the monolayer was again washed three times and covered with 1 ml of Neuman-Tytell medium containing $60 \mu\text{g ml}^{-1}$ soybean trypsin inhibitor. After 60 h the medium was collected and dialysed overnight against 100 mM Tris-HCl pH 4.5 and lyophilized to dryness, and then reconstituted in one-tenth its original volume in 100 mM Tris pH 7.4 containing 5 mM CaCl_2 and assayed for elastase by the azocasein method². The macrophage monolayer was lysed and DNA content measured by a fluorometric assay^{9,10}. Results are expressed as μg equivalents of hog pancreatic elastase activity per μg DNA.

not. A positive control obtained with supernatant from Con A-stimulated spleen cells is shown for comparison (Table 1). These same colonies were screened for their ability to enhance the production of the second complement component by macrophage monolayers³. W25 and W38 enhanced the production of C2 4- and 2.5-fold, respectively, as shown in Table 1. Unfortunately, W25 was unstable and died. W38 was cloned by limiting dilution without feeder layers, resulting in the generation of four clones which were subjected to the same screening procedures.

Table 1 shows the inhibition of migration induced by cloned hybridoma supernatants. In contrast to the colonies, all clones of W38 had migration indices of less than 1 but supernatants from clone C1 appeared even more active than supernatants from Con A-activated spleen cells. Results are given at the dilution of Con A supernatant which gave the greatest inhibition of migration and results were considered positive only if their migration index was ≤ 0.75 (ref. 6). The same supernatants were used to activate macrophages for production of C2. Table 1 shows that the same clone which was most active in the MIF assay gave a twofold enhancement of C2 production.

In two sets of experiments shown in Fig. 1, we measured elastase released into serum-free medium by a macrophage monolayer preincubated with serial dilutions of W38 C1 or control supernatants. In both experiments the supernatant from W38 C1 caused a three- to fourfold increase in production of elastase. DNA contents were not significantly different for either activated or non-activated macrophages, being 0.54 ± 0.098 and $0.545 \pm 0.101 \mu\text{g DNA per ml}$, respectively. W38 C1 supernatants have consistently been negative for interferon.

Our studies are the first to report a factor derived from a T hybridoma which directly affects macrophage function. Our data indicate that both migration inhibitory and macrophage activating activities are present in supernatants from the same cloned T-cell hybridoma. However, the results do not necessarily imply that they are the same molecule; indeed, multiple activities may be present and furthermore our factor(s) may be distinct from those which enhance macrophage cytotoxicity. These questions can be resolved only by chemical charac-

terization of the active moieties present. We are now attempting to do this using macrophage elastase and plasminogen activator as markers for macrophage activation.

Our studies demonstrate that it is possible to preserve lymphokine production by activated spleen cells, by fusion to BW5147 whose supernatant has no biological activity in any of our assays. Establishment of a cell line offers several advantages: (1) production of a continuous level of activity relatively free from other mediators, (2) a homogeneous cell population for studying cell-surface markers, and (3) a source for extraction of specific mRNA.

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Synthesis of hepatitis B surface and core antigens in *E. coli*

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Hepatitis B virus (HBV) is the cause of a debilitating and potentially fatal disease, and possibly also of primary hepatocellular carcinoma¹. A vaccine against hepatitis B would therefore be of considerable biomedical significance. One such vaccine, produced from the 22-nm particle form of hepatitis B surface antigen (HBsAg), has recently been found effective in clinical tests², but being derived from the sera of chronic carriers, it is expensive and its supply limited. The application of recombinant DNA methods can, in principle, provide a limitless source of vaccine. HBV DNA has been cloned in bacteria and the genes coding for HBsAg and hepatitis B core antigen (HBcAg) identified³⁻⁵. Small amounts of both antigens have been synthesized in *Escherichia coli*^{3,6,7} and to enhance this synthesis we have constructed plasmids capable of expressing the genes for the antigens under the control of the efficient tryptophan (*trp*) operon regulatory region⁸. We describe here the construction of such recombinant plasmids which direct the synthesis of high levels of HBcAg and a β -lactamase: HBsAg fusion polypeptide.

The *trp* operon consists of a regulatory region and five coordinately expressed structural genes, *trpE* to *trpA*, respectively (see Fig. 1a and ref. 9). The leader peptide of the *trp* operon (*trpL*)¹⁰ has a *TaqI* site between the Shine-Dalgarno sequence (SD)¹¹ and the initiator AUG codon (Fig. 1b). The location of this site facilitates the construction of a vector suitable for the creation of hybrid ribosome-binding sites, like those described previously for vectors based on the *lac* operon^{12,13}, containing the *trpL* SD sequence and the initiation codon of the foreign gene. Although the synthesis of the 14-amino acid *trp* leader peptide has not been demonstrated, an *in vivo* recombinant between the *trpL* SD sequence and the initiation codon of *trpC* has been shown to direct efficient *trpC* synthesis¹⁴. In addition, some *trp* operon deletion mutants between *trpL* and *trpE* have

been shown to produce *trpL*:*trpE* fusion polypeptides, indicating that *trpL* does contain a functional ribosome-binding site¹⁵.

Plasmid ptrpL1 was constructed from plasmid ptrpE2-1 (see ref. 16 and Fig. 1c) by first isolating the 32-base pair *HpaI*-*TaqI* fragment of ptrpE2-1 by polyacrylamide gel electrophoresis (PAGE). This fragment contains part of the *trp* promoter-operator and the SD sequence of *trpL*¹⁰. The *HpaI*-*TaqI* fragment was recloned into *HpaI*-*ClaI*-digested ptrpE2-1. Because the *TaqI* sequence (T/CGA) of the *HpaI*-*TaqI* fragment is preceded by an A residue on its 5' side, this construction resulted in the formation of a new *ClaI* site (AT/CGAT). The sequence of this junction is shown in Fig. 1d. The resulting plasmid, ptrpL1, has a unique *ClaI* site, three base pairs 3' to the SD sequence of *trpL*, which is useful because several different restriction fragments with 5' CG single-stranded termini can be cloned into it (for example, *HpaII*, *TaqI*, *AcyI*).

Figure 2 outlines the procedure used to construct a series of plasmids that direct the synthesis of HBcAg sequences. A 1,005-base pair *HhaI* fragment of HBV DNA⁵ containing all the HBcAg gene was purified by PAGE and treated with *HpaII* methylase to prevent cleavage of the fragment during linker digestion with *HpaII*. Our HBV DNA clone contains two potential initiation codons for HBcAg⁵. The start site for HBcAg is not known, but most of the evidence is consistent with translation initiation at the second codon⁷. This work utilizes the first initiation codon which should result in the synthesis of a polypeptide containing the entire amino acid sequence for HBcAg and probably an additional 29 amino acids at the N terminus. The initiation codon for HBcAg lies 15 base pairs from one end of this fragment. This fragment was treated with T4 DNA polymerase in the absence of deoxynucleoside triphosphates (to allow the 3' exonuclease to function), in conditions where 0-10 nucleotides are removed from each 3' end, then with *S₁* nuclease. *Bam*HI linkers (CCGGATCCGG, which also contain the *HpaII* site, CCGG) were added and the fragments digested with *HpaII* to generate the 5'CG cohesive end. After purification, these fragments were ligated with *ClaI*-digested and phosphatase-treated ptrpL1. The ligation mixture was used to transform HB101 and recombinants were screened for HBcAg by radioimmune assay (RIA). Seventeen out of 40 recombinants tested were HBcAg positive. Restriction enzyme analysis of the plasmids from HBcAg-positive clones showed that all contained HBcAg sequences and were in the proper orientation for *trp*-dependent expression of HBcAg. Plasmids with the HBcAg gene in the opposite orientation were HBcAg negative.

DNA sequence analysis of four positive clones confirmed that the initiation codon of HBcAg was in close proximity (13-16 base pairs) to the SD sequence of the leader peptide. An HBcAg standard was not available to measure the absolute levels of HBcAg produced in *E. coli*, but the relative levels varied fourfold. The plasmid responsible for the highest level of expression, pCA246, whose sequence is presented at the bottom of Fig. 2, was chosen for further characterization. The amount of HBcAg produced in a 20-min labelling period is 3.2% of the newly synthesized protein uncorrected for cysteine content (~150,000 molecules per cell) as estimated by densitometric scanning of the autoradiogram, see Fig. 3A, lane d. The level of HBcAg can be increased to 10% of newly synthesized protein by optimizing the concentration of the inducer, 3- β indolyl-acrylic acid (IA, unpublished results).

The efficacy of the *trp* promoter in enhancing expression of a linked coding sequence has been demonstrated by the significant production of a fused protein involving the *trpD* gene and human growth hormone coding sequences¹⁷, and by the overproduction of a contiguous β -lactamase gene (Fig. 3A, lane b), which constitutes 12% of labelled protein in cells containing ptrpL1, as a result of partial derepression of *trp* promoters in cells grown in media lacking tryptophan. On addition of IA all *trp* promoters become fully derepressed^{8,9} and the level of β -lactamase increases to 50% of labelled protein (Fig. 3A, lane e).

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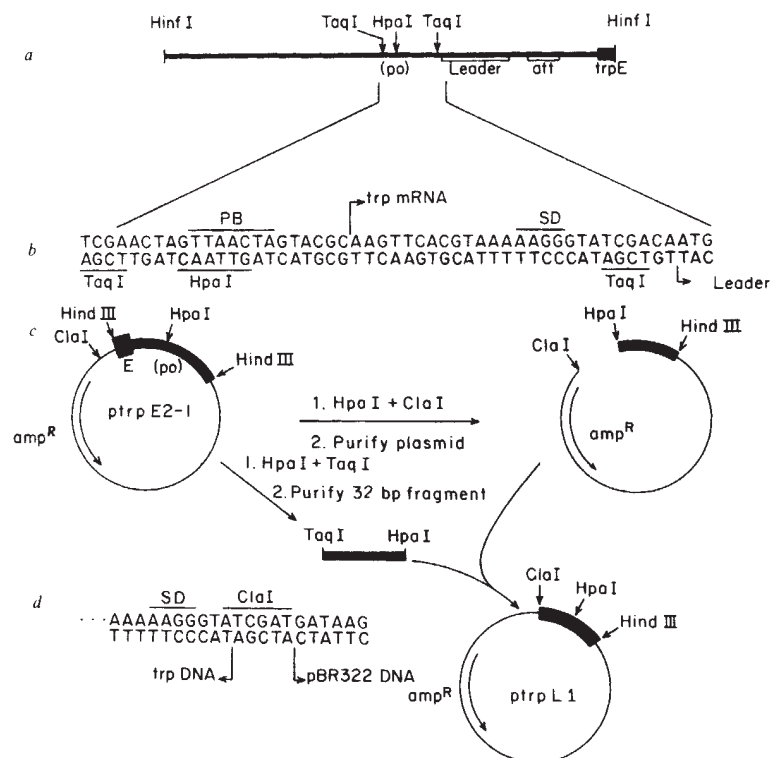
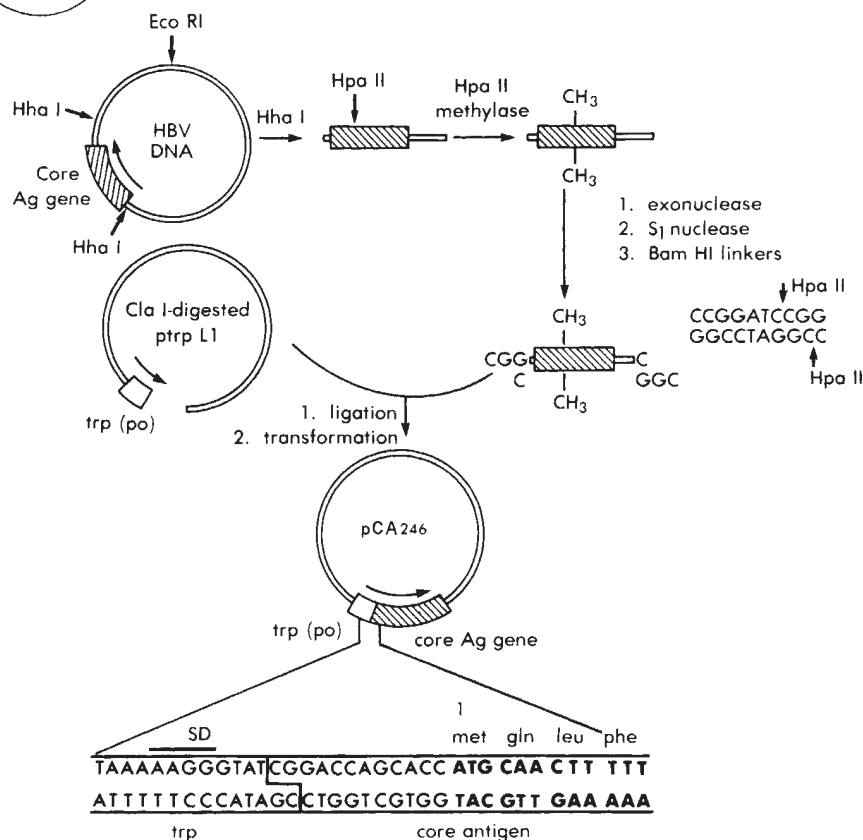


Fig. 1 Construction of expression vector ptrpL1. **a**, Partial restriction map of the 492-base pair *HinfI* fragment of the *trp* operon of *E. coli*. The promoter-operator (po), leader region, attenuator (att) and *trpE* structural gene are shown in their approximate locations¹⁰. **b**, DNA sequence²⁴ of the promoter-operator and proximal portion of the leader region. Note that a *TaqI* site lies between the Shine-Dalgarno sequence (SD) and the initiation codon (ATG) of the leader region. The proposed Pribnow box (PB) and transcription initiation site are also shown. **c**, Construction of ptrpL1. Plasmid vector ptrpE2-1 (construction to be published elsewhere, see text) was digested with *HpaI* and *TaqI* and the 32-base pair fragment of the *trp* operon purified by PAGE^{25,26}. Separately, ptrpE2-1 was digested with *ClaI* and *HpaI* and the plasmid purified by agarose gel electrophoresis²⁶. The plasmid vector (200 ng) was ligated to the 32-base pair fragment (5 ng) in 20 μ l with T4 DNA ligase and the ligation mix used to transform HB101 (ref. 27). Ampicillin-resistant transformants were analysed for plasmids containing single *ClaI* and *HindIII* sites²⁸. Several clones were analysed by restriction mapping and one by DNA sequence²⁹. This plasmid had the expected sequence around the *ClaI* site and was designated ptrpL1. **d**, DNA sequence around the *ClaI* site of ptrpL1 showing the location of the *trpL* SD sequence. Enzymes were purchased from either New England Biolabs or Boehringer-Mannheim and reactions carried out according to manufacturer's instructions.

Fig. 2 Construction of plasmids expressing HBcAg. Cloned HBV DNA⁵ was digested with *HhaI* and a 1,005-base pair fragment containing the HBcAg gene was isolated by PAGE^{25,26}; 20 μ g of this fragment were then treated with *HpaII* methylase³⁰ (a gift from K. Agarwal). After phenol-chloroform extraction and ethanol precipitation the fragment was treated with 28 U of T4 DNA polymerase (prepared by J. Barry) in the absence of dNTPs (to allow the 3' exonuclease activity to function) in 200 μ l of 30mM Tris-acetate, pH 7.8, 67 mM KOAc, 10 mM MgOAc, 0.5 mM dithiothreitol and 100 μ g ml⁻¹ bovine serum albumin for 30 s at 37 °C. The reaction was stopped with phenol, extracted with chloroform and precipitated with ethanol. The fragments were then treated with *S₁* nuclease and *Bam*HI linkers (Collaborative Research) added using T4 DNA ligase³¹. The fragments were digested with *HpaII* and purified from the digested linkers by PAGE and 150 ng were ligated with 200 μ g *ClaI*-digested and calf intestine alkaline phosphatase-treated ptrpL1 (10 U sigma type VII phosphatase incubated with 20 μ g DNA at 68 °C for 30 min in 400 μ l 10 mM Tris, pH 7.5, 0.5 mM EDTA) in 20 μ l and the ligation mixture used to transform HB101 (ref. 32). Transformants were selected on L plates³³ containing 20 μ g ml⁻¹ ampicillin and screened for recombinant plasmid DNA³⁴. Forty such recombinants were then tested for HBcAg using a double antibody RIA with human anti-HBcAg IgG³⁵; 17 were HBcAg positive. Analysis of DNA after digestion with restriction enzymes showed that all 17 contained HBcAg DNA sequences in a *trp* dependent expression orientation. Four were chosen for DNA sequence analysis. The sequence of the plasmid producing the highest levels of HBcAg, pCA246, is shown. The sequence of the others was slightly longer having one or three additional nucleotides of HBV DNA.



When cells containing pCA246 were treated with IA⁹, labelled with ³⁵S-cysteine and the products run on SDS gels, an increased synthesis of a 22,000-molecular weight polypeptide not present in the control strain was observed (Fig. 3A, lanes a, d). The DNA sequence of the HBcAg gene predicts a protein of this molecular weight⁵. In such cells there is also a strong polar effect on β -lactamase production; the decreased level of β -lactamase may be due to a transcription termination site in the HBV DNA or inefficient transcription/translation of HBcAg sequences.

Anti-HBcAg serum precipitates (Fig. 3B, lanes a, b) a 22,000-MW polypeptide, corresponding to the HBcAg protein predicted by the constructed coding sequence, and a polypeptide of MW ~19,000 that is not evident in the labelled proteins resolved on SDS gels (Fig. 3A, lanes a, d). This latter polypeptide probably results from initiation of translation at the second methionine of the 22,000-MW polypeptide or degradation. The pattern of background bands precipitated with anti-HBcAg is different from that in the normal serum control. Sedimentation studies of HBcAg produced in *E. coli* demon-

strate a sedimentation coefficient $>100S$, suggesting that HBcAg is present as some tightly associated complex. As HBcAg functions as a DNA-binding protein³, it may be immunoprecipitated as a complex with DNA or RNA. This would result in the precipitation of other proteins bound to nucleic acids and explain the character of the immunoprecipitates.

It has been suggested that the differential utilization of codons for particular amino acids between *E. coli* and certain eukaryotes¹⁸ may account for the observation that some eukaryotic cDNAs are expressed at lower levels than expected in *E. coli*^{17,19}. One mechanism proposed for this effect is that cognate tRNA molecules are in limiting concentrations for rarely used codons, thus preventing high-level expression of a gene containing several such codons. The HBcAg gene contains 25 arginine codons, 17 of which are rarely utilized in *E. coli*^{5,18}. We have shown that HBcAg is produced in substantial quantities when placed under the auspices of *ptrpL1*. Thus at least in this instance, inclusion of many rarely utilized codons does not prevent high-level expression of a gene sequence in *E. coli*.

Plasmids similar to the HBcAg-expressing ones were constructed using the HBsAg gene. Cells transformed with these plasmids were uniformly negative for HBsAg by RIA. Several clones were analysed by restriction mapping and two by DNA sequence. The distance separating the SD sequence and the initiation codon for HBsAg was 11 and 12 nucleotides. Neither of the clones accumulated polypeptides of the proper size (MW 25,398) on addition of IA, but the polar effect on β -lactamase was still present (data not shown). This result could have been due to rapid degradation or inefficient transcription/translation.

HBsAg contains a prominent hydrophobic region as well as an N-terminal region resembling a modified signal peptide. There are several studies of powerful peptide degradation systems in bacteria including an ATP-dependent system²⁰ probably operative at the cell membrane. We reasoned that elimination of part of the sequence coding for the N-terminal region and subsequent replacement with a nucleotide sequence coding for a stable protein fragment might enhance the stability of this molecule. Because β -lactamase is overproduced in cells containing *ptrpL1*, we elected to try to produce a β -lactamase:HBsAg fusion polypeptide. Figure 4 summarizes the construction of such plasmids. *Pst*I-digested *ptrpL1* was 'tailed' with deoxyguanosine using terminal transferase in conditions where ~ 15 dG residues were added to each 3' end. A 744-base pair *Hinc*II fragment of HBV DNA containing the coding sequence for 204 amino acids (the amino terminal 22 are missing) of HBsAg was purified by PAGE, tailed with dC and hybridized with the tailed *ptrpL1*. The mixture was used to transform *E. coli* HB101.

The DNA sequence of this HBV DNA fragment predicts that when the HBsAg sequences are in the proper orientation and same phase as β -lactamase, a 43,000-MW polypeptide should be produced. This includes 183 amino acids of pre- β -lactamase (MW 20,100), 5–10 glycine residues from the G tail (MW 375–750), and 204 amino acids of HBsAg (22,600). If the pre-sequence is cleaved, the fusion protein would have a predicted MW of 41,000. Thirty-eight ampicillin-sensitive colonies were grown in the presence of IA, labelled, and the products run on SDS gels. Two clones producing a 41,000-MW polypeptide as a major product (Fig. 3A, lanes c, f) were chosen for DNA sequence. As expected, the HBsAg sequences were in the same phase as the amino-terminal β -lactamase sequences (Fig. 4). This fusion protein represents 8.5% of protein (uncorrected for cysteine content) synthesized in a 20-min period ($\sim 170,000$ molecules per cell) as estimated by densitometric scanning of the autoradiogram.

The presumptive 41,000-MW fusion polypeptide is immunoprecipitated with anti-HBsAg IgG (Fig. 3B, lane e). Addition of cold HBsAg inhibits the precipitation completely (Fig. 3B, lane d). Thus, HBsAg determinants are present in the fusion polypeptide. The level of the 41,000-MW polypeptide varied widely in different experiments and in some cases was not detected by

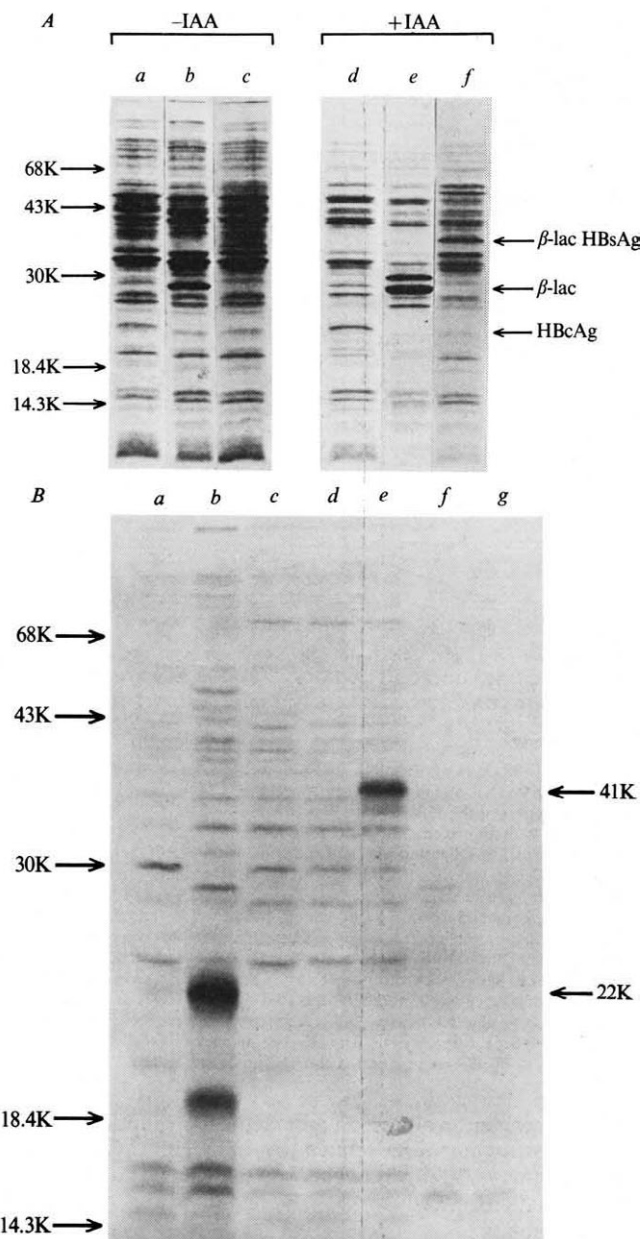


Fig. 3 A, autoradiography of SDS-polyacrylamide gel³⁶ of proteins produced in cells containing pCA246 and pSA4A. Overnight cultures were grown in M9 medium³³ containing 0.25% casamino acids, 0.5% glucose and 0.01% thiamine. The cultures were diluted 1:10 with fresh media, grown for 1 h at 30 °C, $15 \mu\text{g ml}^{-1}$ of IA were added when induction of the *trp* operon was required, and cultures grown for another 2 h at 30 °C (ref. 8). The cultures (1 ml) were then labelled for 20 min at 30 °C with $10 \mu\text{Ci ml}^{-1}$ of ^{35}S -cysteine (NEN), labelled cells collected in 1.5-ml Eppendorf tubes and resuspended by boiling for 2 min in Laemmli sample buffer³⁶. Samples were run on 12.5% Laemmli gels³⁶, the gels were dried and subjected to autoradiography. Lanes a–c are from uninduced cells containing pCA246 (HBcAg), *ptrpL1* and pSA4A (β -lactamase:HBsAg fusion), respectively. Lanes d–f are samples induced with IA of cells containing pCA246, *ptrpL1* and pSA4A, respectively. Molecular weight markers on the left: bovine serum albumin (68,000), ovalbumin (43,000), carbonic anhydrase (30,000), β -lactoglobulin (18,400) and lysozyme (14,300). Arrows on right of the bands denote the migration of the β -lactamase:HBsAg fusion (MW 41,000), β -lactamase (29,000) and HBcAg from pCA246 (22,000). B, autoradiogram of SDS-polyacrylamide gel of immunoprecipitates of HBcAg and β -lactamase:HBsAg fusion produced in *E. coli*. ^{35}S -cysteine-labelled samples were prepared as described above, except labelled cells were resuspended in phosphate-buffered saline containing 1 mM phenylmethylsulphonylfluoride. Cells were sonicated and proteins immunoprecipitated using the SAC technique¹⁷. a, pCA246 + normal human IgG; b, pCA246 + human anti-HBsAg; c, pSA4A + normal guinea pig IgG; d, pSA4A + guinea pig anti-HBsAg and 2 μg unlabelled HBsAg; e, pSA4A + guinea pig anti-HBsAg; f, *ptrpL1* + anti-HBsAg; and g, *ptrpL1* + anti-HBsAg. One μg of each antibody (Merck) was added per tube. Markers on left as above. Numbers on right refer to the β -lactamase:HBsAg fusion (MW 41,000) and HBcAg (22,000) respectively.

mutated in fibrillar muscle to levels comparable with those of actin II. Analysis of the actin pattern in the mutant *rsd* reveals no synthesis of actin III in fibrillar muscle whereas tubular muscle shows the same pattern as in wild-type flies. At the ultrastructural level fibrillar muscles of *rsd* lack thin filaments whereas tubular muscles appear normal. This suggests that the stable actin III found in the wild type represents a form of actin specific for fibrillar muscle.

To detect specific effects of the mutation we analysed tissues with as little contamination by other tissue types as possible^{15,16}. For tubular muscle a preparation of legs with musculature attached—in particular the largest tubular muscle, the tergal depressor of the trochanter—proved to be satisfactory both for handling and for muscle homogeneity. A preparation of the thorax without legs and emptied of internal organs was taken to represent fibrillar musculature (dorsolongitudinal and dorsoventral muscles attached to the cuticle). After *in vitro* labelling these samples were analysed for protein synthesis (by fluorography) and accumulation pattern (Serva blue stain) on two-dimensional gel electrophoresis (Figs 1, 2).

Figure 1 shows that in fibrillar muscle of wild-type flies no actin I is made whereas actin III (and II, which seems to be present in all cell types) is synthesized and accumulated. In contrast, in tubular muscle all three actins are synthesized and accumulated. The accumulation of actin III is less prominent than in fibrillar muscle. Although the data on fibrillar muscle largely confirm the findings of Horovitch *et al.*¹¹, the presence of actin I in tubular muscle contradicts their hypothesis that actin I, detected so far in larval body wall muscles, larval and adult

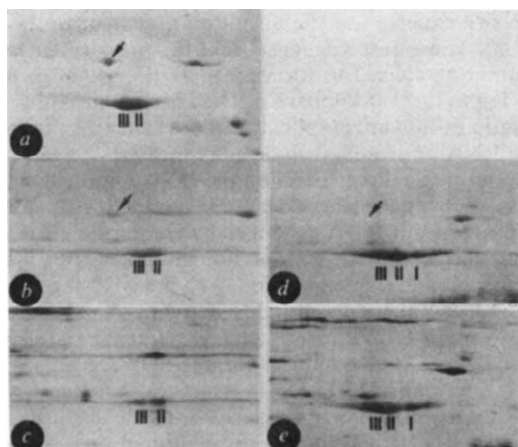


Fig. 1 Analysis of actin accumulation (a, b, d; Serva blue stain) and synthesis (c, e; by fluorography) in adult fibrillar flight muscles (a–c) and adult tubular muscles (d, e) of *Drosophila melanogaster* (Oregon-R). Flies were immobilized on ice and immediately dissected. The samples used were: fibrillar muscles attached to the cuticle of the thorax and tubular muscle of the tergal depressor of the trochanter still attached to the second leg (leg muscles). These were prepared in modified ZH medium²¹, but lacking methionine, yeast extract components and horse serum. For labelling they were transferred to the same medium supplemented with 3 mCi ml⁻¹ ³⁵S-methionine (NEN, 1,108.5 Ci mmol⁻¹) and incubated for 90 min at 26 °C. Sample preparation, two-dimensional polyacrylamide gel electrophoresis (isoelectrofocusing between pH 5 and 8 in the first dimension and 14% SDS-gel electrophoresis in the second dimension), Serva blue staining of the gel and fluorography were performed using published procedures^{2,22,23}. Total protein extracts of fibrillar flight muscles of 5–10 h (a) and 5-day-old flies (b, c) and total protein extracts of tubular muscles of 5-day-old flies (d, e) were separated. For fibrillar and for tubular muscle the samples applied to the gels correspond to the material from 3 and 6 flies respectively. The position of actins I, II and III and of an unknown protein of ~52,000 molecular weight (arrow) are indicated. The actin region of the gels are presented with the more acidic polypeptides to the left.

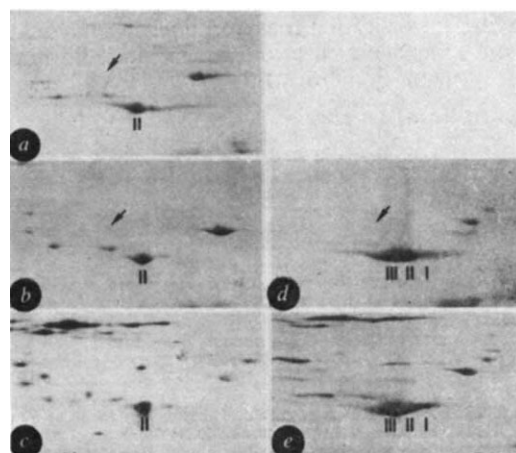


Fig. 2 Analysis of actin accumulation (a, b, d; staining) and synthesis (c, e; fluorography) in adult fibrillar flight muscles (a–c) and adult tubular muscles (d, e) of the mutation raised (*rsd*). Sample preparation and processing and symbols as for Fig. 1. For fibrillar and for tubular muscle the samples applied to the gels correspond to the material from three and six flies respectively. Samples are from newly emerged flies (a) and from 5-day-old flies (b–d).

visceral and adult abdominal muscles, and embryonic myogenic cultures^{11–13}, might be characteristic of supercontracting muscle.

The protein pattern of tubular muscle of *rsd* flies is indistinguishable from that of wild-type flies in both accumulation and synthesis (Fig. 2). When, however, the pattern in fibrillar muscle is analysed, important differences are found. Most obvious is the fact that in fibrillar muscle of *rsd* only actin II can be detected. Because ultrastructure studies show that fibrillar muscle of *rsd* lacks thin filaments (C. A. Reinhardt and A. B. Lang, unpublished data) it may be that in fibrillar muscle thin filaments are normally composed of actin III whereas those of tubular muscle represent actin I. Note that actin III is not even detected as the short-lived component thought to be ubiquitous. If this is so, it must be concluded that the precursor product relationship of actins III and II indicated by the data of Berger and Cox in Kc cells¹⁴ does not hold for fibrillar muscle. It remains an open question whether the electrophoretically resolved actin form III contains more than one protein species—one accumulated in normal fibrillar muscle, another rapidly metabolized in other cell types. These 'two forms' of actin III could conceivably be products of different actin genes, one of them affected by *rsd*.

Some flightless mutants like *heldup-2* (*wingsup A*)^{17–20} or *indented thorax*¹⁹ are known to cause degeneration of flight muscle, so a comparison of two developmental stages (newly emerged and 5-day-old flies) was undertaken. Examination of Fig. 2a, b reveals identical protein pattern for 1-day and 5-day-old fibrillar flight muscle of *rsd*. This indicates that *rsd* does not cause a premature degeneration.

A further protein, of molecular weight 52,000, is also apparently absent from extracts of *rsd* fibrillar muscle. This protein seems to be a specific component of fibrillar muscle because it is also absent in wild-type tubular muscle. Nothing is known about the function of this protein, although it might be identical with a similar, apparently actin-associated component found in muscle of *Lethocerus*²⁴.

Actin gene sequences have been identified by *in situ* hybridization at six sites on the *Drosophila* chromosome^{9,10}. Because none of them corresponds to the genetically mapped site of *rsd* (3–95.4; P. T. Ives cited in ref. 25) and tubular muscle is not affected, we conclude that *rsd* is not an actin gene mutation but rather a tissue-specific regulatory element affecting actin III metabolism. As several other proteins are affected in addition to actin III, it is not clear at what level this regulation occurs.

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An adenovirus mutant defective in splicing RNA from early region 1A

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Many primary products of transcription in eukaryotes contain sequences that are removed during maturation of mRNAs, although the structural determinants that direct this highly specific splicing reaction are unknown. Comparative sequence studies of known splice points¹⁻⁴ have identified a short consensus sequence to which all known splice points conform to varying degrees. However, there is no direct evidence that this consensus sequence is necessary for splicing to occur. In this communication, an adenovirus mutant is described which contains a *cis* dominant defect in the splicing of RNA from one of the early transcriptional units. The defect is ascribed to changes in two adjacent nucleotides within a splice site consensus sequence. The mutations lie in a region of the adenovirus genome (the transforming region 1A) which normally produces a set of three co-terminal mRNAs differing only by the internal sequences removed during post-transcriptional processing. The mutant has thus been used not only to define sequences required for splicing, but also to investigate the pathways along which a series of related mRNAs is generated.

Isolation of the adenovirus type 5 (Ad5) mutant used a screening procedure based on differential viral growth between two human cell lines, 293 and HeLa. The 293 cell line was derived from embryonic kidney cells transformed by fragmented Ad5 DNA⁵. The cells express the left-hand 11% of the adenovirus genome^{6,7} and unlike HeLa cells, support the growth of mutants mapping in this region^{8,9}. The mutants fall into two complementation groups which map to the two transcriptional units in the transforming region, early regions 1A and 1B. To enhance the frequency with which additional such host range (*hr*) mutants could be isolated, a region-specific mutagenesis technique was adopted. This technique took advantage of a phenotypically wild-type variant of Ad 5 (*dl309*) which has a

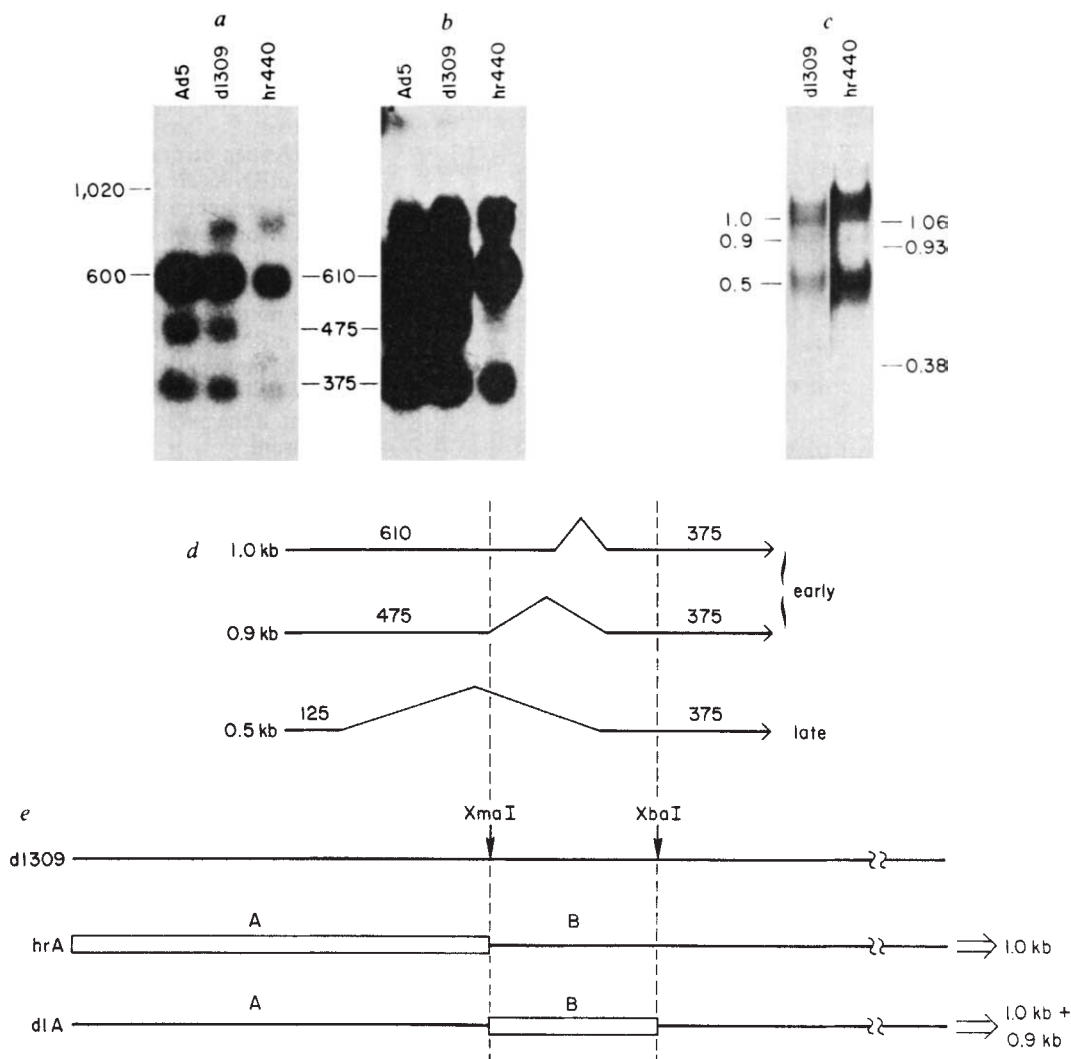
unique *Xba*I restriction site within early region 1A (E1A)⁹. Cleavage of *dl309* DNA with *Xba*I yields two fragments, a small fragment containing most of E1A and a large fragment containing the remaining 96% of the genome (Fig. 1e). The small fragment was mutagenized with nitrous acid¹⁰ and ligated to the untreated large fragment, and the mixture was used to transfect 293 cells⁸. Virus from the resulting plaques was screened for the host-range phenotype. The duration of nitrous acid treatment, 80-120 min, was chosen such that virus from ~20% of the plaques had the mutant phenotype.

One of the mutants, *hr440*, was characterized in detail, with particular attention to the transcription of early region 1A. E1A RNA isolated 9 h after infection of HeLa cells with Ad5, *dl309* or *hr440* was examined using the Berk-Sharp Method¹¹. Figure 1a displays the three separate coding sequences protected from S₁ nuclease digestion by wild-type E1A early RNA¹²: the 610-nucleotide fragment specific for the 1.0-kilobase mRNA, the 475-nucleotide fragment specific for the 0.9-kilobase mRNA, and the 375-nucleotide fragment common to both mRNAs. Using RNA from *hr440*-infected cells, protected fragments comigrating with the 610 and 375 nucleotide bands are again present, but the 475-nucleotide band cannot be detected, even after prolonged exposure of the autoradiograph (Fig. 1b). The absence of the 0.9-kilobase mRNA in *hr440*-infected cells has been confirmed by fractionation of the S₁-resistant RNA-DNA hybrids on a neutral gel (not shown), and by directly displaying the RNA on a formaldehyde-containing agarose gel (Fig. 1c). Furthermore, the 1.0-kilobase but not the 0.9-kilobase mRNA was detected, using the Berk-Sharp method, in nuclear RNA from *hr440*-infected cells. An additional E1A RNA species was seen in both *dl309*- and *hr440*-infected cell nuclei (data not shown). This RNA is 1.1 kilobases long, the size expected of the unspliced precursor to the E1A mRNAs^{7,18}. These results suggest that the primary E1A transcript cannot be spliced to form the 0.9-kilobase mRNA, but that splicing to the 1.0-kilobase mRNA occurs normally.

A mutation could affect splicing either (1) in *trans*, through a diffusible RNA or protein responsible for the splicing event, or (2) in *cis*, through a direct effect on the DNA template or RNA precursor. To distinguish between these possibilities, S₁ analysis was performed using RNA produced by HeLa cells co-infected with *hr440* and *dl313*, an Ad5 deletion mutant lacking most of early region 1B and a small portion (310 bases) of the 3' end of E1A. *dl313* complements mutants mapping in E1A but not those mapping in E1B^{9,13}. Because the deletion extends partially into E1A, S₁-treated hybrids formed between *dl313* RNA and E1A DNA migrate faster in a neutral gel than hybrids formed with *hr440* RNA. RNA derived from each virus in the co-infected cells can therefore be distinguished, so it is possible to determine any effect the co-infection has on the production of individual viral mRNA species. If the mutation were to act in *cis*, the co-infected cells would harbour the same set of E1A mRNAs as the two single infections. If the mutation were *trans*-acting, either the *hr440* 0.9-kilobase mRNA would be present (a recessive mutation), or the *dl313* equivalent of the 0.9-kilobase mRNA would be absent (a dominant mutation). The results of this experiment (not shown) indicate that co-infection has no effect on the production of the individual viral E1A mRNAs; thus, the *hr440* 1.0-kilobase mRNA is present and the 0.9-kilobase mRNA is absent, while the *dl313*-derived equivalents of both these mRNAs are present. The mutation acts in *cis*.

The absence of the 0.9-kilobase mRNA in *hr440*-infected cells, coupled with the *cis*-acting nature of the *hr440* mutation, suggest the possibility that one or both of the splice points used in the synthesis of this mRNA has undergone a mutational change. The 3' splice point, however, is common to both the 0.9 and 1.0-kilobase mRNAs, the latter of which is synthesized by the mutant. It therefore seems likely that the mutation maps near the 5' splice site for the 0.9-kilobase mRNA. DNA sequence analysis in this region (Fig. 2a) uncovered two such nucleotide changes. The two mutations are adjacent, and lie

Fig. 1 Analysis of the region 1A RNAs from *hr440* and its derivatives. *a*, Alkaline agarose gel fractionation of S_1 -treated RNA-DNA hybrids. The poly(A)-containing fraction from 500 μ g of cytoplasmic RNA was hybridized to 0.1 μ g of a plasmid (pHEB1) containing region 1A (map coordinates 0.7-4.3) inserted into pBR322. Fragments fractionated on agarose gels were transferred to nitrocellulose²³, hybridized with 32 P-labelled Ad5 DNA²⁴ and autoradiographed. Preparation of cytoplasmic RNA, RNA-DNA hybridization, S_1 treatment and gel fractionation were performed using the methods of Berk and Sharp¹¹. The lengths in nucleotides of the S_1 resistant DNA fragments are shown on the right. Length markers (Ad5 *Hpa*I F and G fragments) are noted on the left. The fragment migrating more slowly than the 610-nucleotide fragment is derived from an RNA species indistinguishable from the 1.0-kilobase mRNA except for a 140-nucleotide extension at the 5' end²⁵. *b*, Longer exposure of *a*. *c*, Formaldehyde-agarose gel fractionation of poly(A)-containing cytoplasmic RNA. The poly(A)-containing fraction from 300 μ g of total cytoplasmic RNA was loaded on to a 1.5% agarose gel containing 6% formaldehyde. After electrophoresis, the RNA was transferred to a nitrocellulose filter (B. Seed and D. Goldberg, personal communication) and hybridized with 32 P-labelled pHEB1 DNA. Lengths of the individual RNA species are marked on the left; DNA size markers are indicated on the right. The DNA markers migrate slightly faster than RNA fragments of comparable length. The lane marked *hr440* was exposed 10 times longer than the lane marked *dl309*. *d*, Diagram of region 1A mRNAs. Caret symbols bracket the sequences removed during mRNA synthesis; arrowheads represent the 3' ends of the mRNAs. Horizontal lines denote the sequences retained in the mRNA; lengths of these sequences are given in numbers of nucleotides. Total lengths of mRNAs are given in kilobases (kb). On the right is noted the stage after infection at which these mRNAs normally appear (from refs^{12,18-20}). *e*, Structure of recombinants generated from *hr440* and *dl309*. Open boxes denote sequences derived from *hr440*; horizontal lines denote sequences derived from *dl309*. The *Xma*I and *Xba*I sites used for the constructions are indicated in proper alignment with the RNA diagrams above. The precise location of the *Xma*I site is shown in Fig. 2a. On the right are noted the E1A mRNA species generated by the recombinants.



within the intron at distances of 5 and 6 nucleotides from the 5' splice site for the 0.9-kilobase mRNA.

This result suggests that these altered nucleotides are important to the splicing process. Lerner *et al.*³ and Rogers and Wall⁴ have recently proposed a model to explain the mechanism of splicing, based on RNA-RNA hybridization between the abundant small nuclear RNA U1 and the consensus sequences at the two splice junctions of a mRNA precursor. The two mutations introduced into *hr440* lie within the consensus sequence at the 5' splice site for the 0.9-kilobase mRNA (Fig. 2b). The changes might therefore be expected to disrupt the proposed hybridization with U1 RNA. Calculation of the predicted hybrid stability¹⁴ indicates that the negative free energy contribution of the RNA-RNA hybrid formed between U1 RNA and the wild-type 5' splice junction is ~ 5.7 kcal mol⁻¹. Interestingly, this value is decreased to 3.6 kcal mol⁻¹ in the mutant. Whether this difference is sufficient in itself to explain the phenotype of the mutant is unclear. The lack of information concerning the *in vivo* configuration of these putative hybrids should be emphasized.

The data presented thus far suggests that the integrity of sequences adjacent to the splice junction is required for splicing to occur. This conclusion, though, must be accompanied by one important caveat. The sequence of the entire intron for the

0.9-kilobase mRNA, along with 125 bases of 5'-flanking coding sequence, has been determined. In the coding region, two additional single-base changes were found in *hr440*, located 46 and 115 nucleotides upstream from the 5' splice junction. Another base change was found 115 nucleotides downstream from the splice junction, roughly in the middle of the intron. To rule out the involvement of these nucleotide changes in the splicing aberration, the isolation of a phenotypically wild-type revertant of *hr440* would be required. Repeated attempts to isolate such a revertant have been unsuccessful, presumably because more than one base change produces the mutant phenotype. To circumvent this obstacle, an alternative strategy was adopted. Viral recombinants were constructed in which a portion of the mutant or parental E1A region was replaced by a corresponding DNA segment from the parent or mutant, respectively. This is essentially a mapping procedure similar to marker rescue¹⁵, except that the recombination is done *in vitro* rather than *in vivo*. In one recombinant (*hrA*), only the sequences including and upstream from the 5' splice junction for the 0.9-kilobase mRNA are derived from the mutant; the remaining sequences are wild-type. In the reciprocal recombinant (*dlA*), the splice junction and upstream sequences are derived from the parental virus, while the remainder of the intron and some 3'-flanking sequences are derived from *hr440* (Fig. 1e).

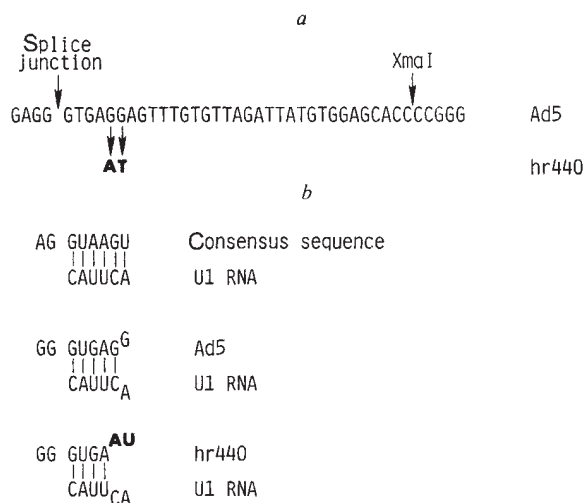


Fig. 2 DNA sequence near the 5' splice site for the 0.9-kilobase mRNA. *a*, Ad5 sequence²⁷ and the nucleotide changes in *hr440*. The *hr440* sequence was obtained by the method of Maxam and Gilbert²⁸, using fragments end labelled at the *Xma*I site. The position of the *Xma*I cleavage site relative to the splice junction is indicated. *b*, Putative RNA-RNA hybrids formed between U1 RNA and sequences adjacent to the 5' splice sites. The mutations in *hr440* are represented in boldface type. U1 RNA and consensus sequences are taken from refs 3 and 26.

The constructions were performed as follows. The small *Xba*I fragment, isolated from both *hr440* and *dl309* DNA, contains a single *Xma*I site located 35 bases downstream from the 5' splice site (Fig. 2a). Cleavage at this restriction site produced an *Xma*I A fragment spanning from the left terminus of the genome to the *Xma*I site, and a B fragment extending from the *Xma*I site to the *Xba*I site near the right end of region 1A (Fig. 1e). The *Xma*I cleavage products were separated and used in two ligation reactions, each containing the large *Xba*I fragment from *dl309* and either (1) the *hr440* *Xma*I A fragment and the *dl309* *Xma*I B fragment, or (2) the *dl309* *Xma*I A fragment and the *hr440* *Xma*I B fragment. The ligation mixtures were used to transfect 293 cells, and virus from the resultant plaques was analysed for host range phenotype and synthesis of E1A RNAs.

Virus derived from ligation 1 (*hrA*) gave a plaquing ratio (titre on 293/titre on HeLa) of 10^4 , which is indistinguishable from that of *hr440*. Virus derived from ligation 2 (*dlA*) showed a plaquing ratio of 70, compared with a ratio of 1 for the parental virus *dl309*. The residual host range exhibited by *dlA* may be due to the mutation located downstream from the 5' splice site which was detected by sequence analysis (this mutation converts an arginine codon to a lysine codon in the coding sequence of the 1.0-kilobase mRNA), or to other unidentified mutation(s) further downstream. Importantly, *hrA*, like *hr440*, produced the 1.0-kilobase mRNA but no detectable levels of the 0.9-kilobase mRNA on infection of HeLa cells; *dlA*, on the other hand, produced both the 1.0- and 0.9-kilobase mRNAs (data not shown). The mutation(s) responsible for the splicing defect thus maps to the left of the *Xma*I site. The only mutational changes in this region which lie in or near the intron for the 0.9-kilobase mRNA are those shown in Fig. 2a. The possibility that the upstream base changes contribute to the splicing defect has not been eliminated but is rendered unlikely by two studies on the splicing of RNA produced from templates deleted of sequences upstream from a 5' splice site. One study showed that the presence of only 18 bases upstream from an intron in the β^{maj} globin gene is sufficient for proper removal of that intron by splicing¹⁶. In the second study, synthesis of an SV40 early mRNA occurred in cells infected by a deletion mutant lacking the region 29–104 nucleotides upstream from the 5' splice junction¹⁷.

At late times after infection, adenovirus produces still another mRNA from region 1A. The structure of this 0.5-kilobase mRNA, as determined by electron microscopy¹⁸, is shown in

Fig. 1d. Comparison of the three E1A mRNAs indicates that they constitute a set of co-terminal RNAs differing only by the positions of the 5' splice sites. Analogous configurations exist in early region 1B mRNAs^{12,18–20}, and in the SV40 early mRNAs^{21,22}. Two simple pathways for the synthesis of these mRNAs can be envisioned: (1) a parallel pathway, in which all three mRNAs are derived directly from an unspliced precursor, and (2) a sequential pathway, where each mRNA is derived as a product of stepwise removal of intervening sequences in the order 1.0 → 0.9 → 0.5 kilobase mRNA. More complex combinations of these two pathways are also possible. The two models make mutually distinct predictions regarding the synthesis of E1A mRNAs, some of which can be tested with *hr440*. If the parallel pathway is used, the 0.5-kilobase mRNA will be present in *hr440*-infected cells even though the 0.9-kilobase mRNA is absent. If the pathway is sequential, the 0.5-kilobase mRNA will be absent.

The presence or absence of the 0.5-kilobase mRNA was investigated using an RNA gel blotting procedure. This procedure was required because, for unknown reasons, the Berk-Sharp method does not detect the 0.5-kilobase mRNA. Cytoplasmic RNA isolated 17 h post-infection was fractionated on a formaldehyde-containing agarose gel and transferred to a nitrocellulose filter. E1A-specific RNA was illuminated by hybridization to a ³²P-labelled E1A probe. The result of this experiment (Fig. 1c) clearly indicates the presence of the 0.5-kilobase mRNA in *hr440*-infected cells. The splicing pathway is therefore not obligatorily sequential. However, the possibility remains that the 1.0- and 0.9-kilobase mRNAs are normally synthesized sequentially, whereas the 0.5-kilobase mRNA is derived in parallel from either the 1.0-kilobase mRNA or the unspliced precursor. Biogenesis of the SV40 early mRNAs also does not require a sequential pathway. Synthesis of the smaller of the two co-terminal early mRNAs (the 16S mRNA) is not affected in cells infected with deletion mutants unable to synthesize the larger (19S) mRNA^{17,29,30}.

Thus we conclude that sequences located within the intron of region 1A and very near the 5' splice junction are required for RNA processing at this splice junction. Furthermore, biogenesis of the three overlapping E1A mRNAs does not proceed in a simple sequential manner. Because the smallest of the three mRNAs is synthesized in the absence of the next larger, this larger RNA cannot be a necessary precursor to the smaller.

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***In vitro* synthesis of polypeptides encoded by the long terminal repeat region of mouse mammary tumour virus DNA**

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The integrated proviral DNA of RNA tumour viruses is bounded by long terminal repeat (LTR) segments of several hundred base pairs which result from duplication of sequences from both the 5' and 3' ends of the viral genome RNA¹⁻³. Recently, DNA sequence analysis of the LTRs of several retroviruses has indicated that this region of the provirus may contain regulatory functions for viral RNA transcription⁴⁻⁸. In this connection, mouse mammary tumour virus (MuMTV) is of particular interest in that transcription of the viral RNA can be specifically modulated by glucocorticoid hormones, suggesting that the site of steroid action may be contained within the LTR⁹. Moreover, the LTR of MuMTV is unusually long, extending for about 1,300 base pairs. We now present data which suggest that, in contrast with other retroviruses, the MuMTV LTR includes approximately 1,000 nucleotides in an open reading frame, capable of encoding a series of polypeptides which are quite distinct from any of the known structural components of MuMTV virions. These proteins can be expressed *in vitro* from cloned, recombinant DNA in which the MuMTV LTR has been inserted into the ampicillin-resistance gene of a bacterial plasmid. Tryptic peptide mapping has confirmed that these products are identical to the analogous series of proteins identified by *in vitro* translation of polyadenylated fragments of MuMTV genome RNA¹⁰.

The ability of certain retroviruses to transform cells in culture and induce rapid neoplasia *in vivo* can be attributed to the expression of specific proteins encoded by sequences within the viral genome. In many instances, these sequences have been shown to be closely related to and possibly derived from analogous elements in normal uninfected cells^{11,12}. This concept cannot, however, fully account for neoplasia induced by the large number of retroviruses, such as MuMTV, which do not seem to transform cells *in vitro* and for which no transformation-specific protein or sequences have yet been identified. To approach this question, we have recently examined the total protein-coding capacity of the genomic RNA by *in vitro* translation and found, as well as the expected translation products derived from the viral structural genes, *gag*, *pol* and *env*, a series of overlapping proteins whose tryptic peptide maps were quite distinct from those of the known structural components of the virus¹⁰. The largest of these (estimated molecular weight MW 36,000 (36K)) would require a coding sequence of about 1,000 nucleotides in a continuous open reading frame. Peptide mapping and limited sequence analysis of the amino-terminal peptides indicated that the smaller components of the series were probably initiated at different methionine codons within the same open reading frame. As no function has been ascribed to these non-structural proteins, we propose to use the term *orf* as an abbreviation for the open reading frame sequences and the products they encode. However, this is not meant to imply that *orf* represents an additional gene.

Translation of subgenomic fragments of MuMTV RNA indicated that the *orf* sequences must be located within the 3'-terminal 2,000 nucleotides of the genome RNA¹⁰. To map the open reading frame more precisely, we have examined the protein-coding capacity of selected regions of MuMTV proviral DNA cloned into bacterial plasmid vectors. As with other

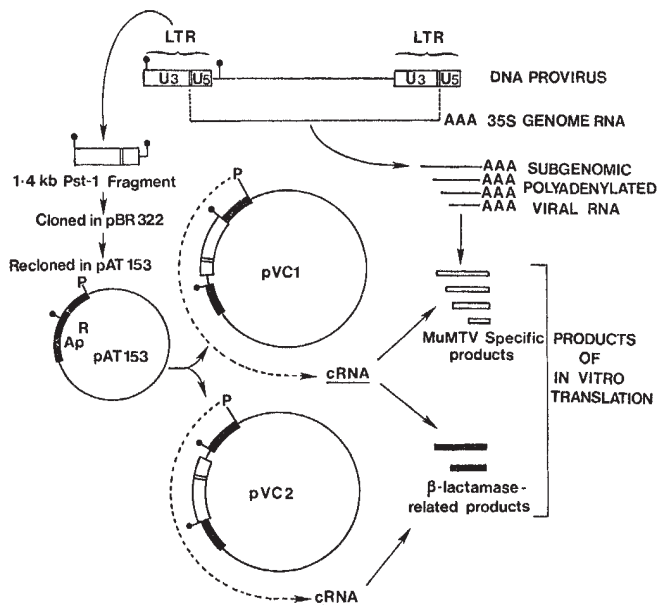
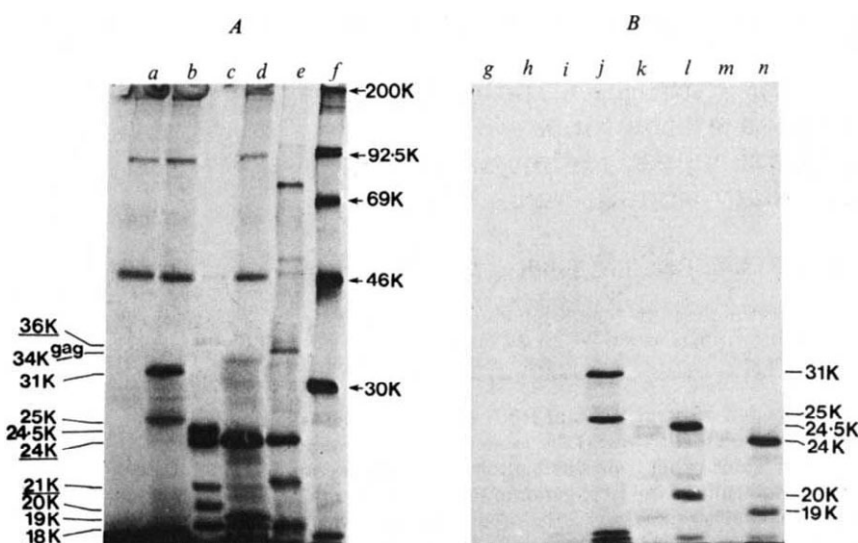


Fig. 1 Construction and *in vitro* expression of recombinant plasmids containing MuMTV DNA. A recombinant plasmid containing the 1.4-kilobase *Pst*I fragment derived from MuMTV proviral DNA inserted into the *Pst*I site of the drug resistance vector pBR322 was originally constructed by Majors and Varmus¹³. The 1.4-kilobase fragment was excised from the plasmid, purified by agarose gel electrophoresis and ligated into the *Pst*I site of the non-mobilizable plasmid pAT153 following standard procedures. Recombinant plasmids were propagated in *E. coli* HB101 and screened for the presence of a single 1.4-kilobase insert of MuMTV DNA. The orientation of the fragment with respect to the promoter (P) for the ampicillin resistance gene (*Ap*^R) was determined by digestion with appropriate restriction enzymes and two representative examples, pVC1 and pVC2, were chosen for further study. Purified plasmid DNAs were used to direct *in vitro* transcription by purified *E. coli* RNA polymerase and the protein-coding potential of the resultant cRNA was assessed by translation in a rabbit reticulocyte lysate as described in Fig. 2 legend.

retroviruses, the genome of MuMTV contains regions at both 5' and 3' termini (designated U5 and U3, respectively) which are duplicated during formation of the integrated proviral DNA³ to give a provirus whose structure resembles that of a bacterial transposable element in that the coding sequences for the *gag*, *pol* and *env* genes are flanked at each end by identical terminal repeats containing both U3 and U5 (Fig. 1)^{4-7,13}. In MuMTV, these LTRs are about 1,300 nucleotides long, of which ~1,200 correspond to the 3' end of the genome RNA (U3)^{13,14}. Cleavage of MuMTV proviral DNA with the restriction endonuclease *Pst*I generates a 1.4-kilobase DNA fragment containing all but eight nucleotides of the left-hand LTR. Using derivatives of a recombinant bacterial plasmid containing this DNA fragment inserted at the *Pst*I site of the drug resistance vector pBR322, we have now examined the protein-coding capacity of the LTR DNA in an *in vitro* system.

In pBR322, and its non-mobilizable derivative pAT153, the cleavage site for the enzyme *Pst*I lies in the coding sequences for the β-lactamase enzyme which confers resistance to ampicillin on recipient bacteria^{15,16}. Transcription of the β-lactamase gene is thought to initiate at a promoter site ~545 nucleotides upstream from the *Pst*I site^{15,17}. We considered that RNA transcripts initiated at this promoter could be extended beyond the *Pst*I site to include any foreign sequences inserted at this position provided that they do not contain transcriptional termination signals. Translation of the resultant RNA in a cell-free system may therefore yield products derived from both the residual β-lactamase gene and any segment of open reading frame within the inserted DNA. Knowing the nucleotide sequence of pAT153, we calculate that the residual part of the β-lactamase gene, from the initiation codon to the *Pst*I site,

Fig. 2 Analysis of products derived by *in vitro* expression of recombinant plasmid DNAs. **A**, Purified plasmid DNAs were used as templates for *in vitro* RNA synthesis in reactions containing 20 mM HEPES (pH 7.9), 100 mM NaCl, 10 mM Mg acetate, 1 mM MnCl₂, 5 mM dithiothreitol, 2.6 mM each of ATP, CTP, GTP and UTP, 1.2 μ Ci ³H-UTP (Radiochemical Centre, 52 Ci mmol⁻¹), 100 μ g ml⁻¹ plasmid DNA and 10–20 units of *E. coli* RNA polymerase (New England Biolabs) in a total volume of 0.12 ml (ref. 19). Following incubation at 37°C for 1 h, the products of the reaction were phenol extracted and ethanol precipitated, and portions taken for *in vitro* translation. Cell-free protein synthesis was carried out in nuclease-treated rabbit reticulocyte lysates primed with 1–2 μ g of plasmid-derived cRNA or MuMTV genome RNA as previously described^{10,18}. The ³⁵S-methionine-labelled products were analysed by electrophoresis in 9% polyacrylamide gels, in the presence of SDS, and visualized by autoradiography: **a**, no RNA; **b**, pAT153 cRNA; **c**, pVC1 cRNA; **d**, pVC2 cRNA; **e**, MuMTV viral RNA; **f**, MW standards. The approximate MWs ($\times 10^3$) of the major products are indicated as computed from their electrophoretic mobilities relative to the standards. Numbers underlined indicate those products not-related to β -lactamase **B**. Aliquots of translation reactions **a**–**d** were immune precipitated using either normal rabbit serum shown in tracks **g**, **i**, **k** and **m**, or anti- β -lactamase serum (anti-TEM-1) as in tracks **h**, **j**, **l** and **n**. Immune complexes were recovered by absorption to *Staphylococcus aureus*, dissociated and the labelled proteins analysed as described above. **g**, **h**, No RNA; **i**, **j**, pAT153 cRNA; **k**, **l**, pVC1 cRNA; **m**, **n**, pVC2 cRNA.



could encode a protein of MW at least 23.9K as compared with 31K for the authentic, full-sized product¹⁵. The precise size of such a truncated product would depend on the location of the first in-frame termination signal encountered in the inserted DNA. Using MuMTV DNA sequences originally cloned in pBR322, two pAT153-based recombinants have been constructed in which the LTR is in a 5' to 3' orientation (pVC1) or vice versa (pVC2) with respect to the direction of transcription from the β -lactamase promoter¹⁵. Purified plasmid DNA was used to direct *in vitro* RNA synthesis using *Escherichia coli* RNA polymerase. Initiation at alternative promoters in the plasmid DNA was suppressed by including 0.1 M KCl in the transcription reactions¹⁷. In the conditions used, the yield of complementary RNA (cRNA) per μ g of input plasmid DNA was 3:1, suggesting several rounds of transcription. The cRNA produced was then purified by phenol extraction and used to programme *in vitro* protein synthesis in nuclease-treated rabbit reticulocyte lysates as previously described^{10,18}. The ³⁵S-methionine-labelled products were analysed by SDS-acrylamide gel electrophoresis and tryptic peptide mapping following standard procedures¹⁰.

The coding capacities of pVC1 and pVC2 DNAs were compared with those of the parental plasmid pAT153 and MuMTV viral RNA (Fig. 2). pAT153 DNA, containing no insert at the *Pst*I site, directed the synthesis of two major polypeptides (Fig. 2A). The larger of these, with an estimated molecular weight of 31K, was assumed to be the authentic primary translation product of the β -lactamase gene, while the smaller 25K product probably reflected initiation of translation at an internal methionine codon within the β -lactamase gene. Both these *in vitro* products could be immune precipitated with an antiserum against the purified protein (anti-TEM-1 serum) (Fig. 2B), confirming that they are indeed related to β -lactamase. In contrast to the parental plasmid, pVC1 DNA directed the synthesis of six major polypeptides of which only two (MWs 24.5K and 20K) were related to β -lactamase as determined by immune precipitation. Thus, the other products, designated 36K, 24K, 21K and 18K, were probably encoded by the MuMTV DNA sequences in the plasmid. As they could not be precipitated by the anti- β -lactamase serum, they probably represent the products of independent, internal initiation events within additional open reading frame sequences provided by the insertion of MuMTV DNA. In pVC1, the inserted MuMTV DNA is in the correct orientation for transcription of the

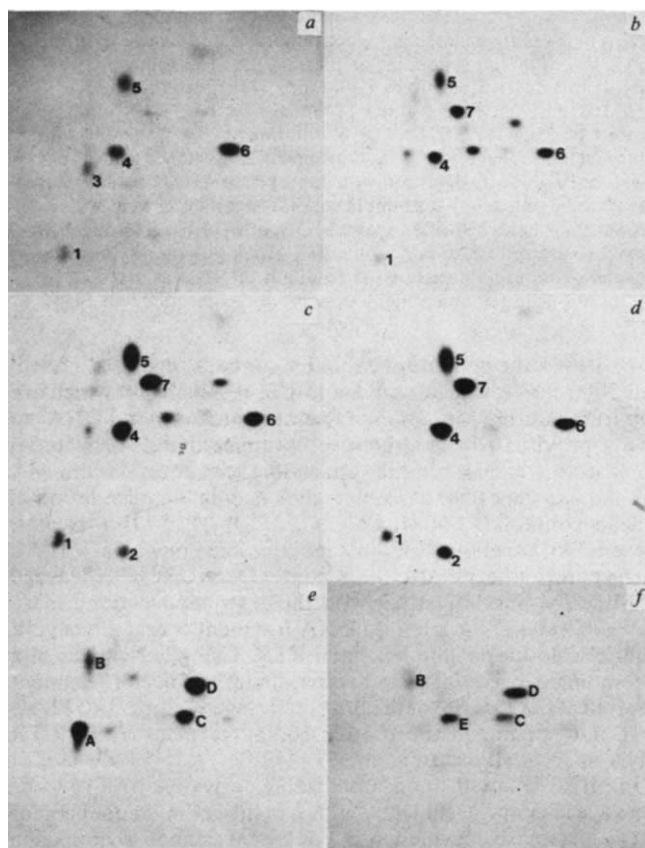


Fig. 3 Tryptic peptide mapping of methionine-labelled products translated from plasmid DNA and MuMTV viral RNA. Polypeptide products identified as bands on the autoradiograph shown in Fig. 2A were excised, eluted from the gel and subjected to performic acid oxidation and trypsin digestion as previously described¹⁰. The labelled peptides were then separated in two dimensions by electrophoresis and chromatography, and visualized by autoradiography¹⁰. **a**, 36K product from pVC1 cRNA; **b**, 24K product from pVC1 cRNA; **c**, mixture of equivalent amounts of the 24K products shown in **b** and **d**; **d**, 24K product from MuMTV viral RNA; **e**, β -lactamase-related 24.5K product from pVC1 cRNA; **f**, β -lactamase-related 31K product from pAT153 cRNA.

positive sense strand from the β -lactamase promoter. With pVC2 DNA, where the LTR is in the reverse orientation, no MuMTV specific products were detected and the two major polypeptides obtained (MWs 24K and 19K) were both immune precipitated by anti- β -lactamase serum. Thus, the negative sense strand of the MuMTV DNA contains no significant stretches of open reading frame detectable in the *in vitro* expression system.

An important feature of the additional products encoded by pVC1 DNA was that their size correlated almost exactly with that of members of the *orf* series of proteins translated from MuMTV viral RNA (Fig. 2Ae). The viral RNA also programmed the synthesis of products from the structural genes, including a 34K *gag* peptide which migrates very close to the 36K *orf* product¹⁰. However, as no antiserum against the *orf* proteins is available, we could not verify this relationship by immune precipitation. To circumvent this problem, the various products obtained by *in vitro* expression of plasmid DNA were eluted and further analysed by tryptic peptide mapping. As expected, the fingerprints of the 36K, 24K, 21K and 18K products from pVC1 represented an overlapping series in that most peptides were shared by each member of the series (see Fig. 3a, b). Peptide analysis also indicated that the pVC1-encoded proteins were virtually identical to those of the corresponding *orf* products obtained by translation of viral RNA (Fig. 3c, d)¹⁰, and substantiated the previous indications that the immune-precipitable products observed in Fig. 2B were derived from the β -lactamase gene (Fig. 3e, f).

We therefore conclude that approximately 1,000 of the 1,300 nucleotides of the MuMTV provirus LTR are organized into a continuous open reading frame which we have termed *orf*. The exact location of the *orf* sequences in relation to the ends of the cloned fragment of MuMTV DNA used in these studies is unknown, but several preliminary conclusions can be drawn. As the *orf* proteins were originally detected by *in vitro* translation of the 3' end of the viral RNA, the coding sequences cannot include the U5 portion of the LTR nor the additional sequences distal to U5 in the cloned *Pst*I fragment, and must therefore be wholly or partly contained within the U3 segment of the genome. As the *Pst*I fragment used to construct the recombinant plasmids lacks eight nucleotides from the 5' end of the LTR, it is possible that the *orf* sequences extend beyond the *Pst*I site and that the linkage of MuMTV to plasmid DNA has occurred within the open reading frame. Thus, initiation of the largest *in vitro* product from pVC1 cRNA may have occurred within the β -lactamase gene, but in a different reading frame, and fortuitously given rise to a 36K species. However, no major differences were observed between the methionine-labelled tryptic peptides of the LTR DNA- and viral RNA-encoded 36K products, and both contained a unique peptide not present in the 24K products or other members of the *orf* series (see Fig. 3a, b and ref. 10). This would imply that at least the major part and probably all of the 36K coding sequences are located within U3.

The U3 regions of several retroviruses, including MuMTV, have now been completely sequenced and shown to contain elements presumably involved in initiation of transcription and polyadenylation of the viral RNA^{4-8,13,14}. In contrast to the other retroviruses, the MuMTV LTR was also found to include a substantial region of open reading frame. However, on the basis of the published sequence data, this reading frame would be insufficient to encode the complete 36K *orf* product, although it could encompass the 24K and smaller *orf* products¹⁴.

Drug resistance plasmid pBR322 containing the MuMTV LTR fragment was provided by Drs J. Majors and H. E. Varmus and anti-TEM-1 serum by Dr M. Boulton (Glaxo).

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Species variation in the specificity of ribulose biphosphate carboxylase/oxygenase

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The balance between photosynthesis and photorespiration in many species, including most crop plants, is determined by the kinetic properties of ribulose-1,5-bisphosphate (RuBP) carboxylase/oxygenase^{1,2}. Photosynthesis is initiated by the carboxylase activity³ while the oxygenase activity catalyses the first reaction in the photorespiratory pathway^{1,2,4}. In these reactions, CO₂ and O₂ are competitive substrates². Because O₂ inhibits carboxylation, and photorespiration oxidizes reduced carbon to CO₂ with no known benefit to the plant, it has been suggested that photosynthetic efficiency and thus productivity might be increased by chemical or genetic alterations of the enzyme which increase carboxylation or decrease oxygenation⁵⁻⁷. On the other hand, it has been argued that RuBP carboxylase/oxygenase cannot completely discriminate between CO₂ and O₂, so that photorespiration is unavoidable⁸. From analyses of RuBP carboxylase/oxygenase enzymes purified from several different species, we report here large differences in specificity towards the substrates CO₂ and O₂. Evolutionary pressures seem to have directed the enzyme towards more efficient utilization of CO₂.

The ratio of carboxylation to oxygenation catalysed by RuBP carboxylase/oxygenase is described by the equation²:

$$v_c/v_o = (V_c K_o / V_o K_c) ([CO_2]/[O_2])$$

where v_c and v_o are the velocities of carboxylation and oxygenation, respectively, V_c and V_o the maximal velocities of the two reactions, and K_c and K_o the Michaelis constants for CO₂ and O₂. The substrate specificity factor, $V_c K_o / V_o K_c$, determines the relative rates of the two reactions at any given CO₂ and O₂ concentrations; a high specificity value indicates a greater specificity for CO₂. We recently devised a method in which both enzyme activities can be assayed simultaneously⁹, and which, when used at several [CO₂]/[O₂] values, permits the direct determination of $V_c K_o / V_o K_c$. Data obtained from applying this assay to enzymes purified from six species are shown in Fig. 1. The specificity factors and relevant kinetic constants for these and enzymes from nine additional sources are summarized in Table 1. The specificity factors ranged from about 12, in enzymes isolated from anaerobic photosynthetic bacteria, to about 80, in enzymes isolated from higher plants. In other plant groups the specificity factors increased from about 48 in two cyanobacterium species to 54 in *Euglena gracilis* to 62 in two species of green algae.

Table 1 Specificity factor and component kinetic constants for RuBP carboxylase/oxygenase purified from several species

Species	Specificity factor ($V_c K_o / V_o K_c$)	K_c (μM)	K_o (μM)	V_c/V_o (ratio)
C₃ plants				
<i>Glycine max</i>	82 ± 5	9	430	1.7
<i>Tetragonium expansa</i>	81 ± 1	13	600	1.8
<i>Spinacea oleracea</i>	80 ± 1	14	480	2.3
<i>Lolium perenne</i>	80 ± 1	16	500	2.6
<i>Nicotiana tabacum</i>	77 ± 1	11	650	1.3
C₄ plants				
<i>Amaranthus hybridus</i>	82 ± 4	16	640	2.0
<i>Zea mays</i>	78 ± 3	34	810	3.3
Green algae				
<i>Scenedesmus obliquus</i>	63 ± 2	38	660	3.6
<i>Chlamydomonas reinhardtii</i>	61 ± 5	29	480	3.7
<i>Euglena gracilis</i>	54 ± 2	25	410	3.3
Cyanobacteria				
<i>Aphanizomenon flos-aquae</i>	48 ± 2	105	990	5.1
<i>Coccolithus pericystis</i>	47 ± 2	121	1,220	4.7
Photosynthetic bacteria				
<i>Rhodospirillum rubrum</i>	15 ± 1	89	406	3.3
<i>Rhodopseudomonas sphaeroides</i> II	9 ± 1	80	220	3.3
<i>Rhodopseudomonas sphaeroides</i> I	62 ± 4	36	840	2.7

Enzymes were purified and specificity factors were determined as described in Fig. 1 legend. For K_c and K_o determinations, the proteins were preincubated at 4 °C for 4 h in 10 mM NaH¹⁴CO₃ (2 Ci mol⁻¹), 10 mM MgCl₂ and 50 mM Bicine at pH 8.5. Assays (30 s) were initiated by adding 100 μg or less of activated protein in 20 μl to reaction mixtures at 25 °C containing 0.40 mM RuBP, 10 mM MgCl₂ and 50 mM Bicine at pH 8.22 or 8.30 in a total volume of 1.0 ml. NaH¹⁴CO₃ concentrations varied between 0.7 and 15 mM, and O₂ concentrations were 0, 0.62 and 1.24 mM. The reactions were stopped by adding 0.5 ml of 3M formic acid in methanol. After drying the samples, ¹⁴CO₂ incorporation was determined by scintillation spectroscopy. K_c was estimated from a Scatchard plot; K_o , determined as the $K_i(\text{O}_2)$, was estimated from Dixon plots and from secondary plots of $K_i(\text{O}_2)$ (apparent) as a function of CO₂ concentration. Lines were fitted by least-squares analysis. V_c/V_o was calculated from the relationship: $V_c/V_o = \text{specificity factor} \times (K_c/K_o)$. All experiments included *S. oleracea* enzyme as a control. Standard errors in the specificity factor determinations are listed. The mean standard error for K_c was $\pm 7\%$ of the value, and $\pm 12\%$ for K_o .

Rhodopseudomonas sphaeroides contains two RuBP carboxylase/oxygenase enzymes¹⁰: one is a lower-molecular-weight protein which, like the enzyme from *Rhodospirillum rubrum*, consists of a single subunit type; the other is a higher-molecular-weight protein similar to the enzyme comprising eight large and eight small subunits obtained from green algae and higher plants. The low-molecular-weight protein (designated II in Fig. 1 and Table 1) was kinetically similar to the *R. rubrum* enzyme, whereas the high-molecular-weight protein (I) was kinetically similar to the algal enzymes. These correlations suggest that high CO₂ specificity requires both subunit types.

The major cause of variation in the specificity factor was changes in K_c , as reasoned previously⁶. However, substantial differences in K_o and V_c/V_o were also found (Table 1). The low-molecular-weight enzymes from photosynthetic bacteria had a low affinity for CO₂ and a high affinity for O₂. The increased specificity factor in cyanobacteria was caused by a decreased affinity for O₂. The affinity for CO₂ was greater in green algal and higher plant enzymes than in bacterial enzymes by up to an order of magnitude, while the affinity for O₂ was intermediate to that found in enzymes from photosynthetic and cyanobacteria. Variation in the ratio of the maximal velocities of the two reactions was also found, ranging from V_c/V_o values of about 2 in C₃ plants (those which fix CO₂ by the Calvin cycle reactions) to about 5 in the cyanobacteria.

These observations suggest that RuBP carboxylase/oxygenase has evolved to compensate, at least in part, for the geological shift from an atmosphere containing high CO₂ and

low O₂ concentrations to one consisting of low CO₂ and high O₂ levels, a shift occasioned by the appearance of O₂-evolving photosynthesis¹¹. The specificity for CO₂ in algal and cyanobacterial enzymes is not as high as that observed in enzymes from C₃ plants, perhaps because the unicellular species have developed mechanisms unknown in detail which concentrate CO₂ internally by a factor of 10 or more¹². The resulting high internal CO₂ concentration reduces the need for a more efficient enzyme compared with C₃ species, in which the carboxylase is in relatively free equilibrium with the external atmosphere. C₄ plants (those which initially fix CO₂ by the enzyme phosphoenolpyruvate carboxylase) also concentrate CO₂ (ref. 13), yet enzymes from these plants have a high specificity factor (Table 1). C₄ species were derived from C₃ species¹⁴, so that high CO₂ specificity of the C₃ enzyme seems to have been conserved despite the generally higher K_c values in C₄ compared with C₃ enzymes¹⁵.

The data presented here demonstrate that the balance between RuBP carboxylation and oxygenation is not immutable, and that modifications increasing carboxylation efficiency and the affinity for CO₂ have occurred during the course of natural history. In the context of plant productivity, the question

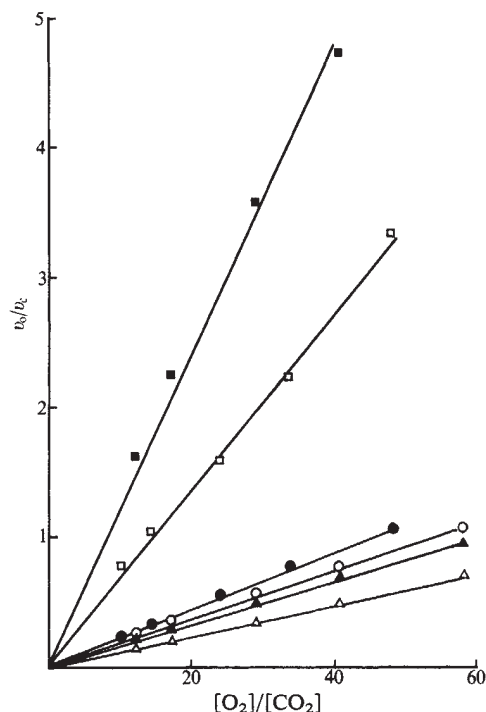


Fig. 1 Determination of the substrate specificity factor. RuBP carboxylase/oxygenase from the various species was purified by a combination of gel filtration and ion-exchange chromatography using Sepharose 6B, Sephadex G-200 and DEAE-Sephacel. Following overnight dialysis at 4 °C against 20 mM Bicine at pH 8.0 and 0.1 mM EDTA, RuBP carboxylase and oxygenase activities were determined simultaneously as previously described⁶. Reaction mixtures containing 2.5 mM NaH¹⁴CO₃ (2 Ci mol⁻¹), 10 mM MgCl₂, 50 mM Bicine at pH 8.22 or 8.30, and ~50 μg of protein were incubated for 10 min at 0, 0.26, 0.37, 0.62, 0.87 or 1.24 mM O₂ in a total volume of 0.5 ml. Reactions were initiated with 10 nmol of [1-³H]RuBP (30 Ci mol⁻¹) and stopped after 5 min with 0.1 ml of a solution containing 0.5N H₂SO₄ and 0.05 M ZnSO₄. Labelled products of the carboxylase reaction (³H, ¹⁴C]glycerate-P) and the oxygenase reaction (³H-glycolate-P) were separated following glycolate-P hydrolysis and radioactivity in these compounds was quantified by scintillation spectroscopy. The ratio (v_o/v_c) of the two reactions, which is independent of RuBP concentration and enzyme activation state⁹, was then plotted against the ratio of the O₂ and CO₂ concentrations present during assay. The specificity factor $V_c K_o / V_o K_c$ is the reciprocal of the slope of this plot. Data is plotted for enzymes from *R. sphaeroides* (II) (■), *R. rubrum* (□), *A. flos-aquae* (●), *E. gracilis* (○), *S. obliquus* (▲) and *S. oleracea* (△).

still to be answered is whether further increases of the efficiency of the enzyme can be accomplished by the application of suitable selection pressure in combination with mutagenesis⁷ or by other techniques.

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Modification of calmodulin function by enzymatic carboxyl methylation

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Calmodulin is a ubiquitous calcium-binding protein that regulates a variety of enzymes such as adenylate cyclase, cyclic nucleotide phosphodiesterase, ATPase and protein kinase (for review see ref. 1). So far, no enzymatic modification of calmodulin has been shown to affect its function. Another ubiquitous protein, the enzyme protein-carboxyl methylase (PCM), modifies proteins post-translationally by methylating their free carboxyl groups and thus neutralizing negative charges^{2–6}. This enzyme is one of three elements of the protein-carboxyl methylation system; the two others are the substrates, the methyl acceptor proteins (MAP) and the demethylating enzyme, protein methyltransferase (PME)⁷. We report here that calmodulin is an excellent good substrate for PCM and that the enzymatic post-translational methylation of calmodulin inhibits its stimulatory effect on cyclic nucleotide phosphodiesterase. Furthermore, we present evidence that carboxyl methylation of calmodulin occurs in intact cells.

Bovine brain calmodulin was purified essentially according to Watterson *et al.*⁸ with the exception that the acid-ammonium sulphate step was omitted. Methyl group (0.2 to 0.5 mol) was incorporated into calmodulin during 30 min at 37 °C in the following incubation medium: 0.5 µg calmodulin, 0.25 M sodium acetate pH 6.0, *S*-adenosyl-methionine 75 µM, 0.1% Triton X-100 and 2,000-fold purified PCM⁹ in a final volume of 50 µl. The extent of methylation was determined in parallel samples by adding *S*-adenosyl-[Me-³H]methionine to the incubation mixture, precipitating the calmodulin-³H-methyl esters formed with 15% trichloroacetic acid, hydrolysing the ³H-methyl esters with 1M sodium borate buffer, pH 11.0, and counting the radioactive methanol formed⁹. As a control, calmodulin or PCM or *S*-adenosyl-methionine was omitted from the incubation mixture. Calmodulin was an excellent substrate for PCM (Table 1). On a molar basis, methylation of

Table 1 Comparative carboxyl-methylation of various purified proteins

Proteins	d.p.m. per pmol of protein
Calmodulin	1,140
Luteinizing hormone	684
Ovalbumin	201
Follicle-stimulating hormone	110
Catalase	143
Carboxylesterase	77
Bovine serum albumin	71
Aldolase	17
Haemoglobin	9
Cytochrome c	3
Myoglobin	3

About 1 µg of each protein was methylated as described elsewhere⁹ using *S*-adenosyl-[Me-³H]methionine as methyl donor. Protein concentration was determined according to Lowry *et al.*¹⁶ using bovine serum albumin as a standard. Values are the mean of duplicate determinations in a single experiment, representative of three others.

calmodulin was 60–70% greater than that of luteinizing hormone, one of the best substrates known¹⁰. Polyacrylamide gel electrophoresis of methylated calmodulin in acid urea shows only one peak of radioactivity that co-migrates with calmodulin (Fig. 1).

To assess the effects of methylation on calmodulin function, samples were assayed using a calmodulin-free preparation of cyclic nucleotide phosphodiesterase. As protein-methyl esters are unstable at neutral and alkaline pH^{11,12}, the phosphodiesterase assay described by Kincaid *et al.*¹³ was performed at pH 6.0. In these conditions, the activation of cyclic GMP hydrolysis was similar to that observed at pH 7.5–8.0 with a 5–10-fold stimulation at optimum calmodulin and calcium concentrations.

Enzymatic carboxyl-methylation reduces the capacity of calmodulin to activate phosphodiesterase (Table 2) and the

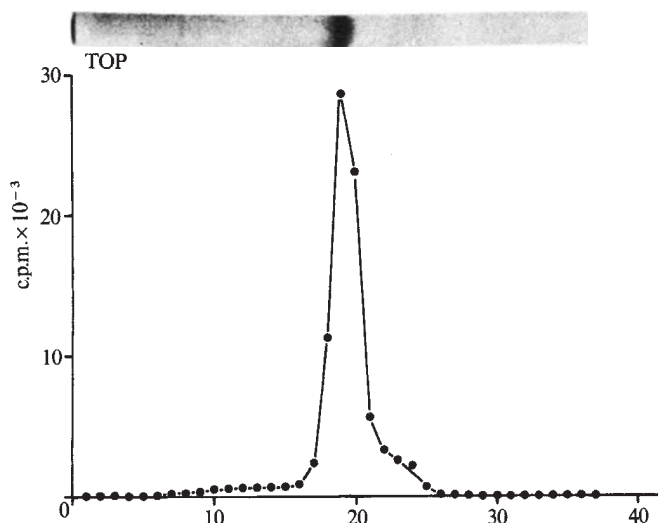


Fig. 1 Polyacrylamide gel electrophoresis of methylated calmodulin. Methylated calmodulin (10 µg) was subjected to electrophoresis on 10% polyacrylamide gel in an acetic acid-urea system as described elsewhere¹². Gels were stained, destained, sliced and counted. The quantity of radioactivity present in the calmodulin band was identical to that recovered as [Me-³H]methanol in a similar sample processed by the trichloroacetic acid method, which indicates that all radioactivity incorporated was in the form of methyl esters. Control gels (not shown), for example, protein-carboxyl methylase alone, reveal only one faint Coomassie blue-staining band at 20% of the gel length with no radioactivity detected in the whole gel.

degree of reduction correlates with the extent of methylation. Incubation of calmodulin in the methylating system from which PCM or *S*-adenosyl-methionine was omitted did not alter its capacity to activate phosphodiesterase (data not shown).

To determine whether the carboxyl methylation of calmodulin is simply an *in vitro* reaction or represents a biochemical reaction occurring *in vivo*, cells from two different cell lines were incubated with [*Me*-³H]methionine. After a 3-h labelling period, a calmodulin-enriched fraction was isolated by phenothiazine-Sepharose affinity chromatography and analysed on acetic acid-urea gel. Two peaks of protein-³H-methyl esters were observed on the electrophoretogram (Fig. 2). Whereas the identity of the first peak of radioactivity is unknown, the second, major peak corresponds to calmodulin as judged from the migration position of authentic calmodulin.

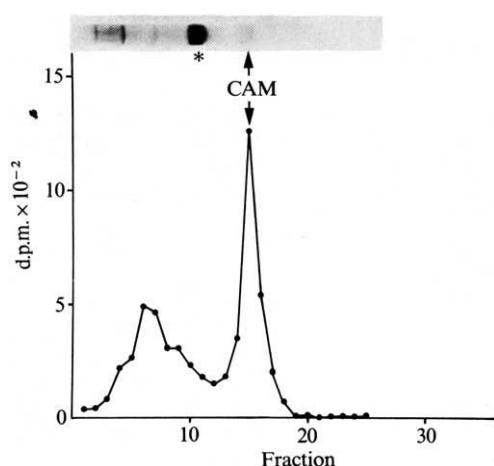


Fig. 2 Polyacrylamide gel electrophoresis of a calmodulin-enriched fraction isolated from intact cells labelled with [*Me*-³H]methionine. Ascitic Walker carcinoma cells (1 g) in Earl's modified minimal essential medium supplemented with 10% fetal calf serum were labelled with 10 μ Ci ml⁻¹ of [*Me*-³H]methionine. After 3 h, cells were sonicated in 0.4 M sodium acetate buffer, pH 6.0, in the presence of 1 mM EGTA and a 100,000 g supernatant was prepared. This supernatant was adjusted to 5 mM CaCl₂ and loaded on to a 2-chloro-10 (3-aminopropyl)-phenothiazine-Sepharose column^{18,19} equilibrated in acetate buffer and 5 mM CaCl₂. The column was washed in the equilibration buffer, followed by acetate buffer plus 1 mM CaCl₂. A calmodulin-enriched fraction was obtained by eluting the column with 10 mM EGTA in acetate buffer. As the chromatography was done in acidic conditions and small volumes of cells were used, only 50% of the original calmodulin (as judged by the phosphodiesterase assay) was recovered in the EGTA fraction. The other 50%, much contaminated by proteins, was recovered with 8 M urea. The EGTA fraction was precipitated with acetone at pH 2 or alternatively lyophilysed after acidification and analysed on acetic acid-urea gel². After electrophoresis, the gels were stained, destained and sliced. Slices were incubated with 400 μ l of sodium borate buffer, pH 11.0, for 6–12 h to allow the hydrolysis of protein-³H-methyl esters to occur. The radioactive methanol formed was extracted using 6 ml of isoamyl alcohol-toluene (2:3 by volume). Two aliquots of 2.5 ml of the organic phase were collected; the radioactivity in one aliquot was counted directly whereas the second aliquot was evaporated to dryness before counting. Normally, over 95% of the radioactivity is volatile. Two peaks of radioactivity are seen on the gel and the second, major peak migrates to the same position as calmodulin. When purified calmodulin is methylated by purified PCM and processed through the phenothiazine-Sepharose column, the recovery of calmodulin-methyl esters in the EGTA plus urea fractions is ~45%. The instability of calmodulin-methyl esters is responsible for this low recovery. Very similar results were obtained with another cell line, the melanoma B16 F10 cells (data not shown). The arrows indicate the position of calmodulin and the asterisk indicates the position of an internal marker, cytochrome c.

Table 2 Effect of enzymatic carboxyl-methylation of calmodulin (CAM) on its capacity to stimulate cyclic nucleotide phosphodiesterase (PDE)

	PDE activation (c.p.m.)	CAM-activated PDE (c.p.m.)	% Inhibition of CAM activation
PDE alone	687		
PDE + CAM (15 ng ml ⁻¹)	1,250	563	
PDE + methyl-CAM (15 ng ml ⁻¹)	1,020	333	41
PDE + CAM (50 ng ml ⁻¹)	1,656	969	
PDE + methyl-CAM (50 ng ml ⁻¹)	1,142	455	53

After the methylation reaction, samples equivalent to 0.6 and 2 μ l were assayed with a calmodulin-free preparation of cyclic nucleotide phosphodiesterase prepared according to Sharma and Wang¹⁷. Phosphodiesterase activity was assayed in a 400 μ l assay containing 0.5 μ M [³H]-labelled cyclic GMP, 5 mM MgCl₂, 0.1 mM CaCl₂, 0.2 M sodium acetate buffer, pH 6.0, 0.1 mg ml⁻¹ ovalbumin, and native or methylated calmodulin (0.51 mol methyl group per mol calmodulin) at the indicated concentration. Mixtures were incubated at 30 °C for 15 min. Values are the mean of duplicated determinations in a single experiment, representative of three others.

These results provide the first evidence that carboxyl methylation of a protein substrate alters its function and of the regulation of calmodulin function as a result of covalent modification catalysed by a ubiquitous enzyme. Moreover, the data suggest that such regulation may be occurring in intact cells. Whether methylation alters the ability of calmodulin to bind calcium or to interact with the phosphodiesterase remains to be answered. Such answers will be difficult to obtain due to the instability of calmodulin-methyl esters at neutral and alkaline pH.

As the recently described mammalian protein methyl-esterase⁹ can hydrolyse calmodulin-methyl esters (unpublished data) synthesized by PCM, the protein-carboxyl methylation system could, by its action on calmodulin, reversibly regulate the activity of the Ca²⁺-dependent cyclic nucleotide phosphodiesterase as well as other enzymes activated by calmodulin.

In mammals, the protein-carboxyl methylation system seems to be involved in white blood cell chemotaxis and exocytotic secretion (for review, see ref. 7), two cellular processes in which calcium has a key role. Both PCM and protein methyl-esterase are activated after exposure of rabbit neutrophils to the synthetic chemoattractant fMet-Leu-Phe^{14,15}. The data presented here suggest that calmodulin is a possible candidate for such regulation.

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BOOK REVIEWS

Probity in science: the case of Cyril Burt

J.M. Thoday

FOR most of the life of the subject of genetics, and longer, students of "heredity" have been faced with two controversies, each of which has had political undertones, and sometimes overtones. One concerns the inheritance of acquired characters, the other the inheritance of variation in ability. Debate about the inheritance of acquired characteristics has had its scandals and its tragedies, and is not wholly dead.

The other controversy is very much alive today. And this pamphlet concerns one of its major scandals, for it reports the results of a meeting of the British Psychological Society, called to consider the implications of the evidence that the contributions of Sir Cyril Burt to psychometrics were at least in part based on fabricated data.

Well before this meeting took place, it had become clear that some of Burt's data were suspect if not fabricated. When Kamin first reported evidence of this, reactions seem to have ranged from delight to shock, to sheer disbelief and sometimes anger. Those who reacted in anger (and the Society itself) have of course been accused of a cover-up, but it takes time for a profession to come round to the view that one of its most respected members, on whose data they have relied, was fraudulent.

Be that as it may, most have now come round to the view that all of Burt's data must be treated as suspect and the view is confirmed by Hearnshaw's sympathetic biography (*Cyril Burt: Psychologist* Hodder & Stoughton, 1979; reviewed in *Nature* 282, 150), and in this pamphlet. What remains is to ask how this sort of thing can be prevented in future and how deleting Burt's data affects our answers to the relevant questions about IQ.

I think the clear general message of this pamphlet and of the whole story is that editors of professional journals should be more inclined to publish basic data, and require careful accounts of how raw data have been treated; in addition, as it suggests, editors should be required to submit their own papers to external referees, nominated by someone other than the editor, for many of Burt's publications were not refereed. For the rest we must be grateful to those who, whatever their motives, did the critical research that has shown us Burt's data are not to be relied on. We need vigilance if such questionable data are to be exposed. Mainly it must depend upon the vigilance of the referees and experts. But I think it is at this level that

A Balance Sheet on Burt. Supplement to the *Bulletin of The British Psychological Society*, Vol.33. Edited by Halla Beloff. ISBN 0-901-71513-1. Pp.38. (British Psychological Society: 1980.) £1.

ideological motivation may have a place in science. If strong distaste for scientific conclusions leads to honest and intelligent criticism of the data base or logical base for those conclusions, this is to the good. The trouble is, however, that such criticism tends to be self-defeating, for it is often presented as if the motivation was itself a justification and thus rouses its own antagonists. Hence as the IQ controversy exemplifies, the criticism simply polarizes two antithetical camps, rather than helping to get us closer to the truth.

To turn to what I think is the more important matter: how does the rejection of Burt's data leave the field? That is to say, how do we answer the following questions on the basis only of other data?

(1) *Is relative performance in IQ tests affected by genetic variance, environmental variance, or both, and if both, how much of each?*

I think it is only the last part of this question that is affected by the deletion of Burt's data. His heritability estimates are higher than most. But most estimates, whatever kind of relatives are compared, give correlations between relatives that imply reasonably high heritability, and all imply that heritability is incomplete, as do also adoption studies. This latter has never been in doubt: I do not think anyone has seriously maintained that IQ variance is all genetic, though Burt wished to define "intelligence" as the genetic component.

Of course much will have to be recalculated, and good deal of that which depended heavily on Burt's data will need to be rewritten. But Rowe and Plomin (*Behaviour Genetics* 8, 81; 1978) and Paul (*Behaviour Genetics* 10, 277; 1980) have compared Burt's data with those of other workers and, though Burt's correlations are rather more extreme, only one differs significantly.

We are, therefore, still left with evidence for rather high heritability, in the face of which the only arguments a determined environmentalist can use are to suppose that all the data are spurious, or to rest his case on the effects of environmental correlation between relatives mimicking completely the theoretical genetic correlations. Such environmental correlations must be a difficulty of human

biometrical genetics, but it seems hard to believe that they could explain all the evidence, especially the evidence that the correlation between monozygotic twins reared apart (0.74) is so much higher than dizygotic twins (0.53) or sibs (0.54) reared together.

Such correlations, when assortative mating is allowed for, imply rather high heritability, which, however, is an upper limit, as some part of the correlation will be

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Cyril Burt — what are the consequences of the exposure of his fabricated data?

environmental. But I doubt if the data could be made consistent with a heritability of less than say 0.4, and point out that all the significant "political" issues with respect to within-group comparisons arise qualitatively whether heritability is 0.2, 0.5 or 0.8, and also agree with Clarke's stress that we do not expect different groups to give the same heritabilities.

(2) *What, if any, are the differences or mean IQ between groups (social or racial), and what environmental or genetic components have such differences?*

The removal of Burt's data still leaves the evidence that groups differ. I have spelt out elsewhere the problems of determining the

causes of such differences and the importance of the fact that group distributions overlap, so that stress on the differences of mean is unfair to individuals. This is true whatever the genetic component. Of course it is called scientific racism to have an open mind on the nature-nurture controversy, but I would repeat (see *J. Biol. Educ.* 6, 323; 1972) that there are grave dangers in basing policies such as affirmative action on the assumption that all differences arise from environmental causes and all individuals are of identical potential.

Amongst the matters unaffected by rejecting Burt's data remains the evidence that IQ and social mobility are correlated. Given significant heritability of IQ this implies that part of class differences in mean must become genetic if they are not already so. It is not reasonable to ignore such evidence, whatever one's ideology. I have also pointed out (*J. Biosoc. Sci. Suppl.* 1, 3; 1969) that when group differences involve a morphological marker, as with skin colour, it is at present formally impossible to determine whether any difference of mean distinguishing the groups is all environmental, all genetic or anything in between. But group mean differences would hardly matter if we treated individuals as such, except if correlations between fertility and IQ differed between groups which, as Jensen pointed out, might occur. If so, heritability within the group becomes important as the predictor of future change, and of future mean differences.

(3) *Do IQ tests measure something whose variation matters?*

Some (see, for example, *Rose Symp. Inst. Biol.* 22, 191; 1975) do not think that IQ tests measure anything.

However, these tests rank individuals on a scale just as stature measures do, and differ from stature measures in only three ways: the measurement error may be larger, IQ scales have no zero, and stature measures can be applied equally accurately, or inaccurately, to doors and trousers. But errors in both measurements can be assessed, and the zero point is irrelevant to biometrical analyses since these are solely concerned with deviations from means. The real difference concerns measuring doors and this is what leads many to think IQ tests only measure ability to do IQ tests. But, though we cannot measure educational doors with such tests, there is significant though incomplete correlation with future educational performance, and various other aspects of "success" in life as Terman's investigations first demonstrated. If, therefore, we consider that the attributes of which IQ tests are partial predictors matter, either to the individual or society, then they do measure something that matters. There might be better measures, complementary measures might make more powerful combined predictors. But they do seem to work, and assessment of this is not affected

by the deletion of Burt's data. I therefore tend to disagree with Blackman, a contributor to the pamphlet, whose suggested investigations concerning individual development seem to ignore that the essential issues concern the nature and causes of between-individual variance.

(4) *Should IQ tests, complemented or not, be used in any way?*

This, of course, is the ideologically loaded question and it is not to be answered by a scientist as scientist but only as citizen. Whether they should be used for investigative purposes depends on your belief in knowledge; whether for selective purposes perhaps upon your ideology.

But I would point out that there is an element of fashion in ideology. Dr Gillie says here that "a generation or more of children may have suffered" as a result partly of Burt's efforts. I can only assure him that the editor of this pamphlet is right about the different view that was taken of the 1944 British Education Act in its early days. Many of my generation when young

regarded it as a great socially progressive enactment!

I conclude that Burt's exposure makes very little difference to our knowledge of IQ and its heritability and agree with Anne Clarke when she says in her essay that "I believe that now that Burt's embarrassing results have been disposed of, we can get on with building a solid science of human differences".

In the short run self-criticism is the best insurance against error. Mutual criticism leading to repeat investigation will expose error in the long run, but in an area where conclusions may affect political action, the long run can be too long. In such areas it is particularly important to avoid antagonizing opponents, but to write as carefully and objectively as possible and, if there are data to present, to present them as they are so that others can test them critically. □

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Solar puzzles for radio astronomers

David Layzer

Radio Physics of the Sun. Edited by M.R. Kundu and T.E. Gergely. Pp.475. ISBN hbk 90-277-1120-8; ISBN pbk 90-277-1121-6. (Reidel: 1980.) Hbk Dfl.115, \$60.50; pbk Dfl.55, \$28.95.

THESE well-edited proceedings of an international conference held in August 1979 and sponsored by the International Astronomical Union contain several excellent short reviews, over 50 brief research reports and an edited transcript of the discussion following each communication. Most of the reviews and reports deal with observations of solar radio emission and with the underlying emission mechanisms; a few discuss the radio observations in a broader observational and theoretical context.

In recent years much new information about the quiet Sun, active regions and solar bursts has come from high-resolution microwave observations with large arrays and from observations at metre wavelength with the Culgoora imaging radio telescope in Australia. High-resolution microwave measurements of circular polarization now delineate small-scale magnetic fields in the lower corona. Heliograms at metre wavelengths show the hole (open field-line) and arch (closed field-line) structure so conspicuous in soft X-ray pictures. Sequences of metre-wavelength heliograms have enabled radio astronomers to track moving Type IV solar bursts and measure their polarization, and have shown that the physics of these transients is not as well understood as had been supposed.

Theories of Type III bursts are the subject of a review paper and several brief reports. Certain aspects of the disturbances responsible for these bursts — in particular, how they maintain their identity for so long (tens of seconds) and over such long path lengths (millions of kilometres) — have puzzled theoreticians for nearly 20 years. At this conference, two distinct and mutually inconsistent approaches to these problems are described, one based on quasilinear theory, the other on strong-turbulence theory. Unfortunately, the two approaches are not given an adequate opportunity to confront each other in the recorded discussion.

This volume (and presumably the conference itself) would have been greatly improved by the inclusion of a few introductory and summary reports of sufficient breadth and depth to provide context and continuity. The invited reviews that open each session are excellent, but too short and too limited in scope; and there are no summaries at all. The volume is dedicated to Stefan Smerd. One misses the breadth and depth of the two classic reviews of solar radio astronomy, both published in *Annual Review of Astronomy and Astrophysics*, that he wrote in collaboration with Paul Wild and A.A. Weiss ("Solar Bursts" by J.P. Wild, S.F. Smerd and A.A. Weiss 1, 291-366, 1963; "Radio Bursts from the Solar Corona" by J.P. Wild and S.F. Smerd 10, 159-196, 1972). □

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A matter of some gravity

W. D. Allen

The Search for Gravity Waves. By P.C.W. Davies. Pp.144. ISBN 0-521-23197-3. (Cambridge University Press: 1980.) £5.95, \$13.95.

THERE is no doubt that Joseph Weber's claim in 1969, to have established evidence for gravitational radiation, has, even if unsubstantiated, stimulated great interest. Thus, most reports of the unsuccessful attempts to corroborate Weber have attracted notice in the popular press. Now, Professor Davies's book brings the subject within the range of the intelligent but non-specialist reader. The book has all the hallmarks of a good exposé. There is very little algebra; the author is concerned throughout to make basic principles picturable, and any formulae derived are brought firmly to earth with order-of-magnitude estimates. Where the theory is complex or obscure, Davies directs attention to the variables involved, deduces the form of the equation linking the variables on dimensional grounds and then supplies the constant of proportionality from the theoretical formulae which results from detailed analysis.

Also, the book develops a logical thread of argument throughout, beginning naturally with electromagnetic radiation. This radiation has many parallels with gravitational radiation, and is familiar in everyday life; but there are important differences, which require emphasis. This opening chapter, on electromagnetic radiation, is strongly to be recommended to those who think the subject can be understood only by reference to vector algebra and Maxwell's equations. Next follows the counterpart chapter on gravity waves, which is the core of the book. In particular, considerable space is given to making clear the role of second-order variations in a gravitational field, known to us all in the phenomenon of tides. For gravity waves, both generation and detection depend on these tidal forces. Next, principles become practice. Formulae are developed which show that the radiation power flux density (in SI units) is negligible for any terrestrial gravity wave generator; and is also negligible, at the Earth's surface, for binary stars, the astronomical gravity-wave generators whose output can be accurately calculated. Two other astronomical candidates are discussed; the vibrating neutron star, relic of a supernova explosion, and the collision of two black holes, each of stellar mass magnitude. Given the right distance from the Earth, i.e. the appropriate degree of optimism, and accepting the necessary approximations in the calculations, the terrestrial radiation flux is appreciable. Having established possible terrestrial fluxes, the author turns to detectors. The cross-section for detection of a tonne of aluminium is calculated, and it is shown

that, taking the radiation flux figures given previously, detection is possible.

Finally, the chapter "Have they been seen?" is mainly on Weber's studies, with a brief glance at another experimental methods, and on a description of observations on the slowing down of a binary pulsar. The decay rate of the period closely matches prediction, although alternative explanations need examination.

There are so many points of interest in the book that comment is somewhat random. First, the early disbelief in gravity waves is rather glossed over, perhaps rightly. But one would still like to believe in the authenticity of a remark attributed to a distinguished astronomer in the 1920s: "Gravity waves travel with the speed of thought". Second, the survey of experiments in terrestrial detection is disappointing; there are two experimental groups still active in Britain and available for advice, and a better survey could have been made, even at the expense of a fractional increase in the book's size.

Weber said in the late 1960s that "In the last 15 years, I have learnt much more about Einstein's theory of Brownian motion than I have about Einstein's theory of gravity". This exactly sums up the field of the gravity wave experiments. If, in a future edition, Professor Davies writes as clearly about Brownian motion as he does about radiation, then, irrespective of gravity waves, he will have written an excellent undergraduate text.

I hope this "popular" book has popular acclaim. □

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Lactation reference

Alan S. McNeilly

Hormonal Control of Lactation. By A.T. Cowie, Isabel A. Forsyth and I.C. Hart. Pp.275. ISBN 3-540-09680-9. (Springer-Verlag: 1980.) DM88, \$52.

CONSIDERING the essential role of lactation in the reproductive cycle of mammals, it is surprisingly often neglected in the teaching of reproductive biology. This may in part be due to the absence of a book which reviews the present state of knowledge on the hormonal control of lactation in a comprehensive but straightforward manner. In the past, texts have been either austere tomes or short monographs essentially in the form of notes for students. I believe that the book by Cowie, Forsyth and Hart has now bridged this gap. It is well conceived in leading the reader

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Veljko Veljkovic, Boris Kidric Institute of Nuclear Sciences, Yugoslavia

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through the chemistry of the hormones involved in lactation to chapters on the growth and development of the mammary gland, hormonal control of milk secretion and milk ejection, assays for the hormones and a section on hormonal receptors and their mechanisms. In these it is possible to gain a comparative picture, as far as our knowledge will allow, of the hormonal requirements for mammary development in species as diverse as the Californian leaf-nosed bat, rabbit and monkey. Indeed, the book emphasizes how limited is our knowledge of the endocrine events controlling not only lactation but reproduction as a whole in the vast majority of mammals.

It is satisfying to see that the authors have included the human being in their considerations. Too often in the past has knowledge gained from animals been ignored or remained unknown in clinical practice, as is clearly illustrated in the section on milk ejection. Some 5,000 years ago milkers were aware that an agitated cow did not give milk readily. Similarly, Cooper in 1840 stated that successful breast-feeding also needs a calm and tranquil mind, a point which many busy post-natal wards in the modern hospital

would do well to remember.

It is clear that many of the recent advances in the field covered by this book have resulted from detailed studies using radio-immunoassays of the hormones involved. As the authors discuss, it is salutary to remember that these methods do not necessarily measure the biological activity of these hormones: will our ideas again need changing when true bioactivity is measured?

A minor criticism is the inclusion of many illustrations from the authors' own studies when, in some instances, others would have been more informative. Nevertheless I found this book easy to read, full of invaluable information and in parts enjoyable; for example, one of the authors describes the time when, having previously worked with milligram quantities, he received a bottle containing half a kilogram of crystalline progesterone for studies on the induction of lactation.

This monograph is well presented and should become a standard reference for all those interested in lactation. □

Alan S. McNeilly is a Research Scientist in the MRC Unit of Reproductive Biology, Edinburgh.

What's new in hybridoma technology?

Carlo M. Croce

Monoclonal Antibodies. Hybridomas: A New Dimension in Biological Analyses. Edited by R.H. Kennett, T.J. McKearn and K.B. Bechtol. Pp.423. ISBN 0-306-40408-7. (Plenum Press: 1980.) \$22.50, £18.59.

THE possibility of producing hybridomas secreting monoclonal antibodies of pre-defined specificities has changed the field of immunology considerably and has provided powerful reagents for biomedical investigation. This book is an attempt to summarize the most recent developments in this area of research.

The book is divided into two major sections. The first concerns the production of hybridomas and the many possible applications of hybridoma technology, for example in studies of the structure and the genetics of immunoglobulins, in the study of the cell surface, in the definition of antigens specific for cells of different lineages and for cells at different stages of differentiation, and in the study of human tumour-associated antigens. Considerable emphasis is given to the analysis of the cell surface by the use of monoclonal antibodies. The second and much smaller section is an appendix cataloguing various methods for the production and the characterization of monoclonal antibodies. A short summary of every technique related to the production,

storage and characterization of hybridoma clones is also included.

This book will be of considerable help to investigators who are not familiar with tissue culture and somatic cell fusion techniques and who intend to produce hybridomas secreting monoclonal antibodies for a variety of purposes. It also provides a good overview of some of the most recent applications of hybridoma technology, particularly in the study of the cell surfaces of malignant cells and of cells of different lineages or stages of differentiation. Although the articles are, in general, well presented and informative, they are rather repetitious in that similar studies carried out in different laboratories are described. It would have been much more useful, I believe, if the editors had included only those articles that reflect the "state of the art" in the areas of application of the monoclonal antibodies to biomedical research. They might also have added an overview of some of the more important contributions in this area.

Overall, this volume provides the necessary information to carry out experiments involving monoclonal antibodies and should be an asset to investigators who wish to become familiar with hybridoma technology. □

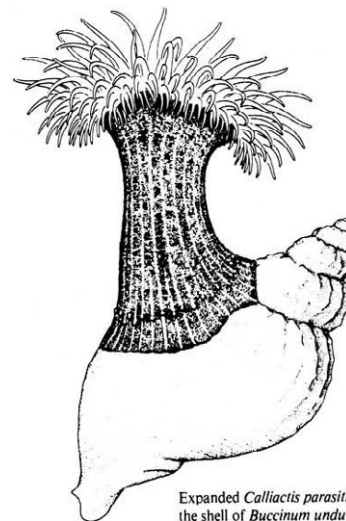
Carlo M. Croce is Associate Director of The Wistar Institute, Philadelphia.

Anthozoan beauty

C.M. Yonge

British Anthozoa. Synopses of the British Fauna, 18. By R.L. Manuel. Pp.241. ISBN 0-12-470050-0. (Academic: 1981.) £7.50, \$18.

THIS is the latest and, next but one, the largest of the *Synopses of the British Fauna*. The series is admirably edited by Doris Kermack on behalf of the Linnean



Expanded *Calliactis parasitica* on the shell of *Buccinum undulatum*

Society in cooperation, in recent parts, with R.S.K. Barnes of the Estuarine and Brackish-water Sciences Association.

While the Anthozoa, in the form of stony, horny and soft corals, flourish most abundantly in shallow tropical waters, the British fauna comprises no less than 73 species. The greatest number are sea anemones, among them six species of the closely related ahermatypic (non-reef-building) corals, three cerianthids (one only known in the larval state) and eight zoanthids. The octocorallines are represented by alcyonarians such as the well-known dead-man's fingers (*Alcyonium digitatum* L.), together with two gorgonids (rock-attached sea fans) and four pennatulids (sea pens) that live unattached but erect in bottom muds.

Most anthozoans, particularly the anemones, are animals of great beauty of colour and delicate design. They have to be described when alive and fully expanded. An extremely high standard of illustration was set by Philip Henry Gosse in *Actinologia Britannica* (1860) and by Alan Stephenson in the two volumes of his Ray Society *British Sea Anemones* (1928, 1935). This book has a wider coverage and, although inevitably denied colour and with only two pages of photographs, the line drawings, largely of living animals and showing pattern and texture, are so beautifully executed as fully to maintain this standard. This is an altogether admirable guide. □

Sir Maurice Yonge is an Honorary Fellow in Zoology at the University of Edinburgh.

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Biological Sciences

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